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How can information on oral bioavailability improve human health risk assessment for lead-contaminated soils?
Implementation and scientific basis

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Rapport in het kort

Hoe kan informatie over orale biobeschikbaarheid de humane risicobeoordeling verbeteren van bodems verontreinigd met lood?
Implementatie en wetenschappelijke basis

Door kennis over het opnameproces van stoffen in het menselijk lichaam beter te benutten, kan de risicobeoordeling van bodemverontreiniging voor de mens verbeterd worden. Inzicht in het opnameproces is verkregen door de nabootsing van het menselijk verteringsproces (*in vitro* digestiemodel).
In dit rapport wordt een concreet voorstel gedaan om de kennis over de opname van lood door het menselijk lichaam in te passen in het nieuwe bodembeleid (Wet bodembescherming). Daarnaast wordt voor risicobeoordelaars en beleidsmakers inzichtelijk gemaakt in welke situaties het zinvol is om met het *in vitro* digestiemodel testen uit te voeren. Naast de toepassing in bodembeleid staat tevens de wetenschappelijke basis van het *in vitro* digestiemodel beschreven. De resultaten van het experimentele model zijn vergeleken met data van de mens en van varkens voor de verontreinigende stof lood om de juistheid van het model aan te tonen.
Voor de toekomst is het van belang dat er internationale harmonisatie plaatsvindt over de toepassing in bodembeleid en de methodiek om kennis over het opnameproces te verkrijgen.

Trefwoorden:
Orale biobeschikbaarheid, bioaccessibility, lood, risicobeoordeling, bodem

Abstract

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By using knowledge on the uptake process of compounds into the human body, the risk assessment of soil contaminants for humans can be improved. Insight into the uptake process is obtained by simulating the human digestion process (*in vitro* digestion model).
In this report a concrete proposal is given for using the knowledge on the uptake of lead in the human body in procedures to assess the soil quality according to the new soil policy (Dutch Soil Protection Act). In addition, risk assessors and policy makers are advised on the situations where performing tests with the *in vitro* digestion model is desirable.
Besides the application in soil policy, the scientific basis of the *in vitro* digestion model has been described. The experimental results of the experimental model have been compared to human and swine data for the contaminant lead to demonstrate the correctness of the model.
In future, international harmonization on the application in soil policy and the methodology to obtain knowledge on the uptake process will become important.

Key words:
Oral bioavailability, bioaccessibility, lead, risk assessment, soil

Preface

Since the late nineteen nineties the National Institute for Public Health and the Environment (RIVM) performed research on bioavailability of contaminants in the human body after oral ingestion of soil ("oral bioavailability"). The purpose of this research was to improve human health risk assessment. In soil quality standards (Intervention Values, Land use Specific Remediation Objectives) and in procedures to assess the site-specific human health risks due to soil contamination the influence of oral bioavailability was neglected. This suggested a potentially significant overestimation of internal exposure and, hence, of the risk to human health. The focus of the research was on lead. The reason for this is that the Intervention Value is exceeded at many sites in the Netherlands, mainly in residential areas, and because of the knowledge that overestimation of internal exposure is relatively frequent. For this reason, research on and implementation of oral bioavailability in human health risk assessment (and human health based soil quality standards) receives a lot of international attention.

In this report, results of 7 years of research are translated into **concrete proposals for implementation of oral bioavailability of lead from soil in the procedures to assess soil quality in the Dutch Soil Protection Act**. Besides, insight in the wider (international) scope of oral bioavailability is given. The scientific background is described and used as the foundation for application of information on oral bioavailability into risk assessment.

Contents

Samenvatting

Dit rapport beschrijft hoe de beoordeling van risico's van bodemverontreinigingen voor de mens verbeterd kan worden door specifieke informatie te gebruiken over de biobeschikbaarheid van een contaminant in het menselijk lichaam na inslikken van verontreinigde bodem (orale biobeschikbaarheid). Het rapport biedt een concreet voorstel voor het verkrijgen van informatie over de biobeschikbaarheid van lood uit bodem, en hoe deze resultaten volgens een getrapte procedure ingepast kunnen worden in de risicobeoordeling. Dit zou op termijn onderdeel uit kunnen maken van het blootstellingsmodel CSOIL.

Aannames

Om tot een realistische risicobeoordeling van lood te komen is in de afleiding van het voorstel om specifieke informatie over biobeschikbaarheid van lood uit bodem in de risicobeoordeling op te nemen uitgegaan van een gemiddeld kind. Dit uit zich in twee belangrijke aannames. Ten eerste is zoveel mogelijk uitgegaan van de gemiddelde fysiologische conditie van een kind. Ten tweede is uitgegaan van een bodeminname van een gemiddeld kind, dat wil zeggen niet van pica-gedrag waarbij bewust grote hoeveelheden bodem worden ingeslikt.

Wetenschappelijke onderbouwing

Voor het verkrijgen van informatie over de biobeschikbaarheid van contaminanten in het menselijk lichaam is de afgelopen jaren door het RIVM een zogenaamd. *in vitro* digestiemodel ontwikkeld. De correlatie van dit *in vitro* digestiemodel met *in vivo* data is beschreven voor lood. De aannames die in de risicobeoordeling worden gedaan zijn zo ver mogelijk in de uitvoering van het *in vitro* digestiemodel verwerkt.

Internationale afstemming

Internationale afstemming ten aanzien van *in vitro* digestiemodellen vindt plaats in ISO (International Standardisation Organisation) kaders, en door overleg met andere instituten waar onderzoek naar biobeschikbaarheid plaatsvindt (onder andere BARGE). Verdere internationale afstemming in het kader van de "EU Soil Strategy" is noodzakelijk om tot een geharmoniseerd Europees bodembeleid te komen.

Nut voor de gebruiker

Op basis van het huidige onderzoek verdient het aanbeveling om de hier beschreven trapsgewijze procedure te gebruiken bij de invulling van het nieuwe bodembeleid. Meer specifiek: bij de afleiding (of onderbouwing) van normen (Interventiewaarden en Referentiewaarden) moet de huidige procedure worden gehandhaafd, dat wil zeggen geen correctie voor biobeschikbaarheid. Eventueel kan voor bepaalde bodemtypes die veel organisch stof bevatten standaard een lagere relatieve orale biobeschikbaarheidsfactor (± 0,5)

worden aangenomen. Bij locatie-specifieke methodieken (Saneringscriterium en Locale Referenties) zou gebruik gemaakt moeten worden van de trapsgewijze procedure waarbij uiteindelijk bij potentieel risico de biobeschikbaarheid locatie-specifiek experimenteel wordt bepaald middels het *in vitro* digestiemodel van het RIVM. De kosten van zo'n bepaling liggen tussen de 175-525 euro per bodem (zie details in hoofdstuk 13). Door het verwerken van locatie-specifieke informatie over biobeschikbaarheid in het menselijk lichaam is de verwachting dat het aantal gevallen van spoedeisende sanering zal verminderen. Na extra onderzoek waarbij de gemiddelde fysiologische conditie van een kind als uitgangspunt wordt genomen kan mogelijk een correctiefactor voor orale biobeschikbaarheid voor generieke risicobeoordeling worden afgeleid.

1. Introduction

According to present soil quality criteria, many sites in the Netherlands should be remediated, which would be an expensive and time-consuming process. In present assessment of risks of contaminated soils for human health some assumptions seem to be unnecessary conservative. Therefore, the RIVM was asked to investigate the possibilities to come to a less conservative and more efficient approach. Human health and other protection targets such as the ecosystem should of course not be compromised. This research fits in the recently announced policy of the Ministry of Housing, Spatial Planning and the Environment (Ministry of VROM, 2003), in which one of the demands was a more efficient attitude towards soil contamination.
One approach that seems promising to come to a more efficient human risk assessment of contaminated soils is accounting for **oral bioavailability of the soil contaminants**.

In the present report, the results of the research are translated into **concrete proposals for implementation of oral bioavailability of lead from soil in the procedures to assess soil quality in the Dutch Soil Protection Act.** Application is proposed for both generic risk assessment (Intervention Values) and site-specific risk assessment. The proposed method to account for oral bioavailability of lead from soil concurs with the methodology of the exposure model CSOIL that is applied in the Netherlands and many other countries worldwide.
The scientific background is described as foundation for the proposed application for risk assessment. A simple method to estimate the oral bioavailability of soil contaminants is described and evaluated.

1.1 Dutch Soil Protection Act

Soil and groundwater quality standards and standardised risk assessment procedures are laid down in the **Dutch Soil Protection Act**. The soil and groundwater quality standards are **generic** (independent of land use), see §1.1.1, whereas the risk assessment procedures enable **site-specific risk assessment**, see §1.1.2 (Swartjes, 1999). The basis for the present soil and groundwater quality standards and standardised risk assessment procedures is the Ministerial letter from 1994 (Ministry of VROM, 1994). More recently, the Ministry of Housing, Spatial Planning and the Environment announced a different approach to soil contamination (Ministry of VROM, 2003). The reasons for this are, among others: the need for a more sustainable approach on soil protection, the demand for a more efficient attitude towards soil contamination and the intended shift towards a more decentralized soil policy. Last, but not

least, the scientific framework of the Dutch Soil Protection Act must be updated. At this moment the revision of the Dutch Soil Protection Act is in preparation.

1.1.1 Generic soil quality standards: Intervention Values

When the total soil concentration exceeds the Intervention Value in a soil volume of at least 25 m^3 there is "a seriously contaminated site", in which case in principle the site has to be remediated. The Intervention Value is a generic, i.e. independent of land use, soil quality standard.

In order to determine the Intervention Value for a contaminant, exposure via different pathways is calculated, for example the exposure via soil ingestion, via the consumption of home grown vegetables, via inhalation of contaminated indoor air, *et cetera*. The so-called standardised exposure scenario representing "an average residential situation" in the Netherlands (Van den Berg, 1995) is applied. The calculation is performed with the exposure model **CSOIL**, see Figure 1, which is used in present risk assessment in the Netherlands (Otte *et al.*, 2001).

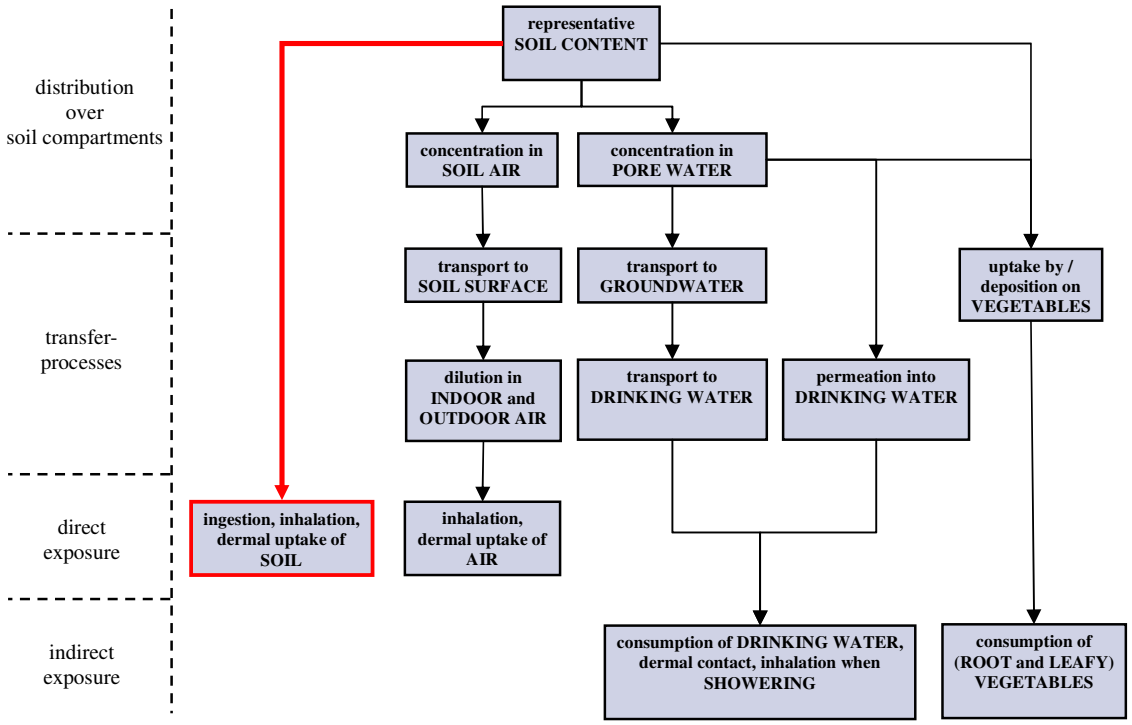

Figure 1. Schematic overview of the different exposure pathways described in the CSOIL model to quantify human exposure to contaminated terrestrial soils (Otte et al., 2001). The present report addresses accounting for oral bioavailability of contaminants after ***direct soil ingestion*** *into risk assessment for humans (see thick red arrow).*

In the next step the exposure from the oral and dermal exposure pathways are summed up and compared with the MPR_{human}. The MPR_{human} is defined as the amount of a substance that any human individual can be exposed to daily during full lifetime without significant adverse health effects (Baars *et al.*, 2001). The concentration in the indoor air is compared with the TCA (Tolerable Concentration in Air) or CR_{inhal} (Cancer Risk Air) (Baars *et al.*, 2001). To be able to incorporate the indoor air pathway in the CSOIL exposure model both the TCA/CR_{inhal} and the exposure to contaminants in air are, in analogy with the oral and dermal exposure and the MPR_{human}, transformed to the unit $\mu g.kg_{body\ weight}^{-1}.d^{-1}$. The Intervention Value based on **human** health risk, also referred to as the Serious Risk Concentration for humans (SRC_{human}), is defined as the total contaminant concentration in soil for which the following risk index equals 1:

$$\frac{\sum \text{oral} + \text{dermal exposure}}{TDI\ or\ CRI} + \frac{\sum \text{concentration in air}}{TCA\ or\ CRA} \qquad (1)$$

Also an Intervention Value based on ecological risk assessment is determined. The Intervention Value that is ultimately used in risk assessment is the Intervention Value with the lowest contaminant concentration based on either human health or ecological risk assessment.

In 2001 proposals for revised Intervention Values were released (Lijzen *et al.*, 2001). At this moment these proposals for Intervention Values have not yet been formalized.

1.1.2 Site-specific risk assessment

Due to the large number of contaminated sites in the Netherlands it is not feasible to remediate all sites within a manageable amount of time and money. For this reason the urgency of remediation of serious contaminated sites has to be determined using a standardised procedure based on site-specific risks (Koolenbrander, 1995). In the present procedure human-toxicological site-specific risk assessment is also based on the MPR_{human} and the CSOIL exposure model. However, major differences between site-specific risk assessment and the derivation of the Intervention Values are that in site-specific risk assessment:

- several standardized exposure scenarios have been included for several land-uses;
- the assessment is based on a tiered approach;
- to improve the reliability of the calculation, measured concentrations in the contact media (indoor air, vegetables) have to be included in higher tier risk assessment.

1.2 Accounting for bioavailability in the human body

In present risk assessment no specific attention is paid to **bioavailability** of contaminants in the human body, neither in regard to generic soil and groundwater quality standards, nor in standardised site-specific risk assessment procedures. More specifically, in present risk assessment **it is implicitly assumed that the bioavailability in the human body of a contaminant from soil equals the bioavailability of the contaminant in the studies on which the MPR_{human} is based.** However, in most studies on which the MPR_{human} is based, the contaminant was present in food or water. The bioavailability of a contaminant in the human body can be considerably lower when the contaminant is in soil, because the contaminant may sorb to the solid phase of the soil during passage through the human gastrointestinal tract. This results in a lower fraction of the contaminant absorbed and thus in a lower bioavailability in the human body and a lower toxicity. Many *in vivo* studies with test animals confirm that bioavailability or toxicity from soil is lower than from food or water (Fries *et al.*, 1989; Freeman *et al.*, 1994; Casteel *et al.*, 1997; Freeman *et al.*, 1996; Schroder *et al.*, 2004). As a consequence, internal exposure to contaminants in soil is overestimated in most cases. However, note that, depending on the matrix, it is also possible that internal exposure is underestimated. Yet, accounting for bioavailability in the human body would result in a more realistic, and in most cases a less conservative approach to human health risk assessment.

By accounting for oral bioavailability, risk assessment is based on **internal exposure** rather than **external exposure** of the contaminant. Internal exposure represents the actual exposure to the contaminant in the human body, i.e. the fraction of ingested contaminant that reaches the central blood circulation. Only this fraction will be able to exert adverse effects. Present risk assessment is based on external exposure, which means that it does not matter in what form or matrix the contaminant is ingested, as long as the total amount of contaminant is the same. For example, the health risk of a contaminant in water or soil is assumed to be the same as long as the total amount of contaminant is the same. By accounting for oral bioavailability, risk assessment is based on internal exposure, and thus better related to the toxic fraction of the contaminant.
The highest bioavailability, i.e. the highest internal concentration, of a contaminant is expected from an aqueous solution for hydrophilic compounds (e.g. metal compounds) or oil for lipophilic compounds (PCBs, dioxins). Bioavailability is assumed to be less from food and lowest from soil, as soil is not degraded in the gastrointestinal tract and has a high adsorption capacity for contaminants.

Considering the arguments mentioned above, research on the oral bioavailability of a contaminant from soil is expected to be relevant. It may lead to a reduction in the calculated risks. Hence, accounting for oral bioavailability of a contaminant from soil in human risk assessment is especially relevant when:

- the soil contaminant gives rise to major problems in current risk assessment;
- there is a potential risk for human health;
- exposure to the contaminant occurs for a significant part via soil ingestion, and
- in the MPR_{human} studies the contaminant was ingested with water, and to a lesser extent, with food.

Information on oral bioavailability of a soil contaminant may be implemented into **site-specific** risk assessment (Remediation Urgency) when experimental knowledge on the oral bioavailability of a contaminant from soil of that specific site is obtained. Information on oral bioavailability may also be used in **generic** risk assessment, i.e. the derivation of Intervention Values, when bioavailability of a contaminant from soil always appears to be lower than from the matrix used in the MPR_{human} studies.

Note that in present Dutch risk assessment of contaminated soil it is assumed that a **child ingests 100 mg soil per day** (Otte *et al.*, 2001). Dutch policy has chosen to estimate the health risks associated to the average behaviour of a child. The 100 mg of daily ingested soil is representative for the average exposure to a child. It does not consider so-called “pica behaviour”, i.e. deliberate soil ingestion by children. Some children have been found to ingest several grams of soil, even up to 60 g, during a single day (Calabrese *et al.*, 1999). At present, very little information is available how many children show “chronic” pica behaviour, i.e. regular pica behaviour, and how much soil is ingested daily in case of chronic pica behaviour.

1.3 Lead

Lead is a contaminant that meets all conditions indicating that accounting for oral bioavailability in human risk assessment of contaminant soil may be relevant (section 1.2). First, Dutch soils are frequently contaminated with lead. In many cases the current Intervention Value is exceeded. Second, exposure to lead is assumed to occur for approximately 70% via soil ingestion (Lijzen *et al.*, 2001). Third, the Intervention Value for lead in soil is based on the MPR_{human}, and there is a potential risk for human health. And finally, the MPR_{human} is based on absorption from dietary lead (IPCS (International Programme on Chemical Safety), 1995; FAO/WHO, 1993; Baars *et al.*, 2001). Hence, accounting for oral bioavailability of lead from soil in the human body is expected to improve risk assessment, i.e. less conservative but still protective for human health.

Note that young **children** are the group with the highest health risk for lead intoxication. First, children ingest larger quantities of soil than adults because they exert hand-to-mouth behaviour (Lijzen *et al.*, 2001; Schmidt, 1999; Davis *et al.*, 1990; Calabrese *et al.*, 1989; Van Wijnen *et al.*, 1990; Reed *et al.*, 1999; Stanek *et al.*, 1998). Soil particles will stick to their hands and when a hand is put into the mouth the soil particles can be ingested. Second, it is

known that children absorb lead better than adults. This assumption originates from various studies which suggest that lead is absorbed by the same mechanism as calcium (Diamond *et al.*, 1997). Calcium is better absorbed in children than in adults as growth demands more calcium (Clarkson, 1993; Fullmer, 1992; Mushak, 1991). Also from a toxicological point of view children are the group at risk since lead already affects children at low doses, resulting among others in impaired neurobehavioural functioning and decreased haemoglobin levels (IPCS, 1995; Baars *et al.*, 2001).

1.4 Aim of the report

The aim of the present report is to recommend how human health risk assessment can be improved by implementation of oral bioavailability of lead from soil. Both the practical implementation into risk assessment and the scientific background are addressed.

To achieve that abovementioned aim, we first searched for an experimental method to estimate the oral bioavailability of lead from soil. For ethical reasons, this method should not use animals. In addition, the method should be relatively cheap and thus simple. Another important issue is that the method should be validated, i.e. gives results that are predictive for oral bioavailability in humans. Subsequently, we investigated whether bioavailability could be estimated from simple soil characteristics so that it can be predicted if the bioavailability of lead from soil is expected to be high or low. We then investigated how the obtained information can be implemented into human health risk assessment. Both site specific risk assessment and generic risk assessment (Intervention Values) will be taken into account. The method to account for oral bioavailability of lead from soil will concur with the methodology of the exposure model CSOIL that is applied in the Netherlands and many other countries worldwide.

1.5 Scope of the report

The present report addresses how information on the oral bioavailability of lead from soil can improve human risk assessment.

Chapters 3 to 10 focus on the scientific background of oral bioavailability of lead from soil. Chapter 11 describes the situation in other countries. Chapter 12 is particularly interesting for policy makers and risk assessors as it discusses the implementation of oral bioavailability into risk assessment and makes recommendations for different levels of complexity (tiers) in risk assessment of contaminated soils. In chapter 13 the conclusions are presented that are of interest for 1) policy makers, local authorities, and risk assessors and 2) scientists.

In more detail, first, the concepts of oral bioavailability, bioaccessibility and relative oral bioavailability are addressed (chapter 2). In chapter 3, the development and description of the RIVM *in vitro* digestion model are presented. This *in vitro* digestion model is a simple tool to estimate the bioaccessibility and oral bioavailability of a contaminant. Other *in vivo* and *in vitro* models to estimate oral bioavailability and bioaccessibility are described and discussed in chapter 4. The validation of the RIVM *in vitro* digestion model with *in vivo* data is described and discussed in chapter 5 for lead, and for several other compounds in chapter 6. Subsequently, in chapter 7, the conditions in the RIVM *in vitro* digestion model are addressed that are recommended for use in risk assessment. Information on the effect of several soil characteristics on bioaccessibility of lead is addressed in chapter 8. The scientific background of chapters 2-8 leads to a practical relationship between the relative oral bioavailability of lead and the bioaccessibility determined with the RIVM *in vitro* digestion model (chapter 9). Whether a default relative bioavailability factors that can be used in the derivation of the Intervention Value of lead in soil is discussed in chapter 10. In chapter 11 the situation regarding oral bioavailability and bioaccessibility in risk assessment in other countries is addressed. Information on oral bioavailability of lead from soil at different levels in risk assessment is applied in chapter 12, resulting in the practical recommendation for implementation of oral bioavailability of lead from soil in risk assessment. A short summary is given at the end of each chapter, and at the end of the report the overall conclusions are summarised in chapter 13.

2. Oral bioavailability and bioaccessibility

For correct implementation of oral bioavailability and bioaccessibility in human health risk assessment, the concepts of oral bioavailability, bioaccessibility, and relative bioavailability should be understood. Below these concepts are explained in detail.

2.1 Concept of oral bioavailability and bioaccessibility

According to the general interpretation in pharmacology, **oral bioavailability is defined as the fraction of an orally administered dose that reaches the systemic circulation**. We have conceptually subdivided **oral bioavailability** (**F**) into three major processes. Figure 2 describes these processes for soil contaminants. After soil ingestion, the contaminants may be partially or totally released from the soil during digestion in the gastro-intestinal tract. The fraction of the contaminant that is mobilized from soil into the digestive juice, i.e. chyme, is defined as the **bioaccessible fraction** ($\mathbf{F_B}$). This fraction is considered to represent the maximum amount of contaminant available for transport across the intestinal epithelium. $\mathbf{F_A}$ represents the fraction of bioaccessible contaminant that is transported from the lumen across the intestinal epithelium and into the portal vein or the lymph. The contaminants may be metabolized in the intestinal epithelium or the liver, which is referred to as the first-pass effect (and they may be excreted).
The fraction of contaminant after the liver without being metabolized ($\mathbf{F_H}$) will be transported throughout the body by the systemic circulation, and may exert toxicity in organs and tissues. Consequently, the orally bioavailable fraction of soil-borne contaminants is the resultant of the three steps: bioaccessibility, transport across the intestinal epithelium, and the first-pass effect (see Figure 2 and equation 2):

$$F = F_B \times F_A \times F_H \qquad (2)$$

The matrix in which the contaminant is ingested, i.e. food, water or soil, is a determining factor in the fraction of the contaminant that becomes bioaccessible. The matrix in which the contaminant is ingested may also affect the absorption of the contaminant. For example by competition for absorption carriers or routes between the contaminant and food components. The absorption of lead is influenced by the presence of calcium in the matrix, as lead is supposed to use calcium absorption channels (Diamond *et al.*, 1997; Clarkson, 1993; Fullmer, 1992; Mushak, 1991). In many inorganic compounds, however, the matrix does not influence the metabolism of the contaminant.

Note that is it is possible that the fraction that is available for transport across the intestinal epithelium is underestimated. This may occur if transport across the intestinal epithelium is fast and the equilibrium between bioaccessible and non-bioaccessible contaminant is disturbed and additional delivery of the non-bioaccessible to the bioaccessible fraction occurs. This is only expected if transport across the intestinal epithelium is fast. In practice, for compounds that are not very soluble, this is only expected in case of active transport across the intestinal epithelium.

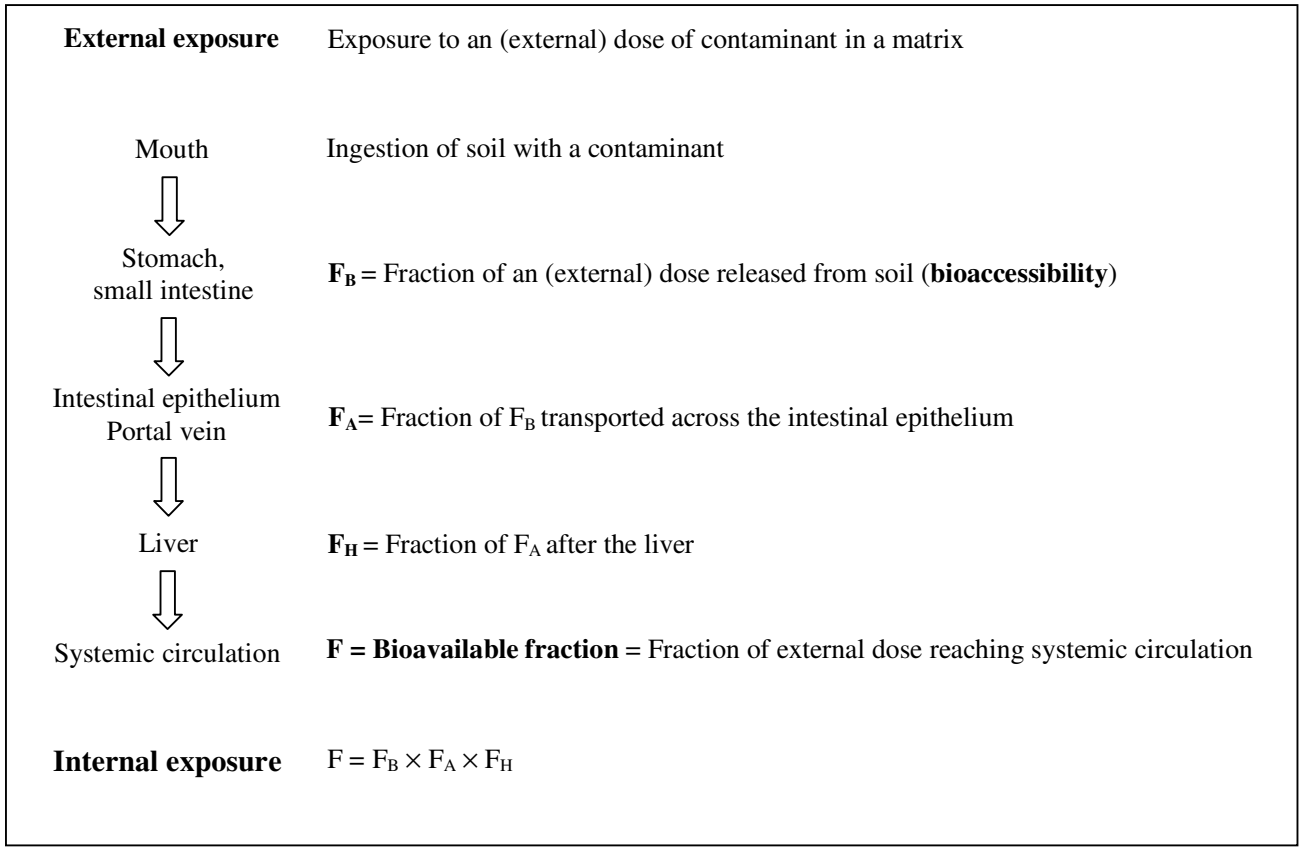

Figure 2. Various steps of oral bioavailability (F) of a contaminant from soil.

2.2 Concept of relative bioavailability

In present risk assessment it is assumed that **the bioavailability in the human body of a contaminant from soil equals the bioavailability of the contaminant in the studies on which the MPR_{human} is based.** However, in most studies on which the MPR_{human} is based, the contaminant was present in food or water. The bioavailability of a contaminant in the human body can be considerably lower when the contaminant is in soil, because the contaminant may sorb to the solid phase of the soil during passage through the human

gastrointestinal tract. This results in a lower fraction of absorbed contaminant, and thus in a lower bioavailability in the human body and a lower risk on adverse effects.

The difference in bioavailability of a contaminant between soil and the matrix in the study on which the MPR_{human} is based can be quantified by a **relative bioavailability factor** (*Rel* F).

$$Rel\ F_{contaminant} = \frac{F_{contaminant\ from\ soil}}{F_{contaminant\ from\ matrix\ MPR\text{-}human}} \quad (3)$$

The exposure model CSOIL already describes a relative bioavailability factor (Otte *et al.*, 2001). In CSOIL, exposure of lead by a human being via ingestion of soil (and house dust) is calculated according to:

$$DI = \frac{AID \times C_s \times Rel\ F}{W} \quad (4)$$

With:

DI:	uptake via ingestion	($mg_{contaminant} \times kg^{-1} \times d^{-1}$)
AID:	daily intake soil/house dust via ingestion	($kg \times d^{-1}$)
W:	body weight	(kg)
Rel F:	relative bioavailability factor, presently set at 1	(-)
C_s:	Concentration contaminant in soil/house dust	($mg_{contaminant} \times kg^{-1}$)

At the moment, this relative bioavailability factor is always put on “1”, i.e. the difference in bioavailability between soil and the matrix used in MPR_{human}-studies (water, food) is not quantified.
In order to account for oral bioavailability in risk assessment, information is required on the oral bioavailability of a contaminant from the matrix used in the studies upon which the MPR_{human} is based relative to the bioavailability of the contaminant from soil.
For lead, dietary lead was the matrix of ingestion in the studies upon which the MPR_{human} is based. Hence, for lead the following **relative bioavailability** can be derived:

$$Rel\ F_{lead} = \frac{F_{lead\ from\ soil}}{F_{dietary\ lead}} \quad (5)$$

Oral bioavailability is considered to be the product of bioaccessibility, absorption and metabolism, see Figure 2. As lead is not metabolised, i.e. $F_{H,soil} = F_{H,MPR} = 1$, the relative bioavailability of lead can also be described as:

$$Rel\ F_{lead} = \frac{F_{B,soil} \times F_{A,soil} \times F_{H,soil}}{F_{B,MPR} \times F_{A,MPR} \times F_{H,MPR}} = \frac{F_{B,soil} \times F_{A,soil}}{F_{B,MPR} \times F_{A,MPR}} \quad (6)$$

Hence, with information on the bioaccessibility of dietary lead ($F_{B,MPR}$) and of lead from soil ($F_{B,soil}$), and information on the absorption of lead ($F_{A,soil}$ and $F_{A,MPR}$), the relative bioavailability (*Rel* F_{lead}) can be estimated. The bioaccessibility might be determined in a simple, fast and cheap manner without the use of test animals. Therefore, a tool for the assessment of bioaccessibility in the human gastrointestinal tract has been developed to obtain information on the relative bioavailability in a simple, fast and cheap manner. This tool, an *in vitro* digestion model, is described in detail in chapter 3.

2.3 Conclusion

The orally **bioavailable fraction** of a compound is the fraction that reaches the systemic circulation (= blood stream), and can exert adverse effects. Oral bioavailability can be subdivided into three major processes:

- Bioaccessibility
- Absorption
- Metabolism

The **bioaccessible fraction** is the fraction that is mobilised from its matrix (e.g. soil, food, water) in the human gastrointestinal tract, and becomes available for intestinal absorption. In present risk assessment, it is assumed that the bioavailability in the human body of a contaminant from soil equals the bioavailability of the contaminant in the studies on which the risk assessment is based, which was usually food or water. However, the bioavailability of a contaminant from soil is mostly lower than the bioavailability of the contaminant from water or food. The difference in bioavailability of a contaminant from soil versus the matrix used in the studies upon which the risk assessment is based can be quantified by the **relative bioavailability factor**. Implementation of the relative bioavailability factor in risk assessment is expected to lead to a more realistic and less conservative estimation of the exposure to a contaminant after soil ingestion.

3. The RIVM *in vitro* digestion model

We aimed at developing a tool for estimating oral bioavailability of a contaminant from a certain soil sample or other matrix in order to make an estimate of the relative bioavailability factor. It would be very expensive and time consuming to determine the oral bioavailability of each soil sample by *in vivo* studies. Moreover, it limits the possibility to test various exposure scenarios. An *in vitro* model was therefore preferred over *in vivo* studies. Conform Figure 2 in chapter 2, this means that the test should simulate the process of bioaccessibility. Since the matrix of ingestion (soil versus water and food) is the main cause of the difference in bioavailability of a certain contaminant from soil versus water or food, this sub-process of bioavailability was chosen to simulate with an *in vitro* test. Hence, as a tool for estimation of the bioaccessibility, an *in vitro* digestion model was developed that simulates the physicochemical conditions of the human gastrointestinal tract.

In the present chapter, the development and procedure of the RIVM *in vitro* digestion model are described.

3.1 Development of the *in vitro* digestion model

The *in vitro* digestion model introduced by Rotard *et al.* (1995) was used as a starting point for the experimental design of the RIVM *in vitro* digestion model. Both the Rotard model and the RIVM model are static gastro-intestinal models. Digestive juices are prepared artificially. The composition of the digestive juices is based on human physiology. The digestive juices are added to a soil sample according to physiological transit times and are mixed thoroughly. The rationale for choosing the number of simulated compartments of the gastro-intestinal tract, temperature, soil-to-fluid ratio, ratio of digestive juices, transit times, centrifugation, pH values, mixing, constituents and their concentrations, and bile, are addressed in Oomen *et al.* (2003a).

Within the development of the *in vitro* digestion model, extra attention was paid to the choice of bile. When the *in vitro* digestion model was first developed, freeze dried chicken bile was used as bile component. The reason for using chicken bile was that the model that was used as a starting point for the *in vitro* digestion model of the RIVM also used chicken bile (Rotard *et al.*, 1995; Oomen *et al.*, 2004b). However, in 1999 the supplier stopped the sale of freeze dried chicken bile. Another bile type had therefore to be chosen. The animal origin of bile may give rise to differences in bioaccessibility because bile composition appears to be species dependent. Therefore, the bioaccessibility of benzo[a]pyrene, arsenic, cadmium, and lead from four different soils after digestion with ox bile from two different suppliers, pig

bile, and chicken bile was studied. Only chicken bile increased the bioaccessibility of lead and cadmium significantly and relevantly for one of the four soils. The bioaccessibility of lead was 3 to 5.5 times greater, and the bioaccessibility of cadmium was 1.5 times greater, for chicken bile than for the other bile types. In all other cases, the bioaccessibility differences were less than 10%, which is considered irrelevant for risk assessment purposes. Hence, ox or pig bile is preferred to chicken bile in *in vitro* digestion experiments because:
1. Chicken bile may lead to an irregular and unaccountable bioaccessibility pattern.
2. The composition of chicken bile is very different from the composition of human bile.
3. The percentage of a specific bile salt in human bile is in almost all cases an intermediate of ox and pig biles.
4. Ox and pig bile lead to similar percentages that were bioaccessible for all soils and contaminants tested.
Ox bile was used for further use in the *in vitro* digestion model. Further details on the effect of bile on bioaccessibility can be found in Oomen *et al.* (2004b).

The *in vitro* digestion model developed within the present project simulates fasted conditions of the human gastrointestinal tract. Within another project, an *in vitro* digestion model was developed that simulates fed conditions of the human gastrointestinal tract, where it was used to assess the bioaccessibility of certain contaminants (mycotoxins) from food. Differences in physiology between fasted and fed state may give rise to differences in bioaccessibility, as pH, salt and enzyme concentrations are different. In the present research, the digestion model simulating fed condition was used to study the bioaccessibility of lead from soil for fed conditions, i.e. the physiological conditions shortly after consumption of a meal. The development of the *in vitro* digestion model simulating fed conditions is described by Versantvoort *et al.* (2004; 2005). The model simulating fed conditions has been used occasionally for lead-contaminated soils.

3.2 Description *in vitro* digestion procedure

Below the procedure of the *in vitro* digestion is described for both fasted and fed conditions.

3.2.1 Fasted conditions

A schematic representation of the *in vitro* digestion model for fasted conditions is presented in Figure 3. The digestion starts by introducing 9 ml of saliva of pH 6.5 ± 0.2 to 0.06 or 0.6 g of soil (dry weight). This mixture is rotated head-over-heels for 5 minutes at 55 rpm. Subsequently, 13.5 ml of gastric juice (pH 1.07 ± 0.07) is added, and the mixture is rotated for 2 hours. The pH of the mixture is measured. The mixture of saliva and gastric juice usually has a pH of about 1.2, and the allowed pH interval in the presence of soil is 1.5 ± 0.5. Finally, 27 ml of duodenal juice (pH 7.8 ± 0.2) and 9 ml bile (pH 8.0 ± 0.2) are added

simultaneously, and the mixture is rotated for another 2 h. The pH of this mixture with intestinal juices is measured. The allowed pH interval is 6.0 ± 0.5, also depending on the soil. All digestive juices are heated to 37 ± 2 °C. Mixing is done in a rotator that is also heated to 37 ± 2 °C. At the end of the *in vitro* digestion process, the digestion tubes are centrifuged for 5 minutes at 3000 g, yielding the chyme (the supernatant) with and the digested soil (the pellet).

3.2.2 Fed conditions

The *in vitro* digestion simulating fed conditions starts by introducing 0.04-0.4 g of soil (dry weight) to 6 ml stimulated saliva (pH 6.8 ± 0.2) and 4.5 g infant formula (product number 282, Olvarit (Nutricia®, the Netherlands), supplemented with 2 ml sunflower oil per 100 g). This infant formula with sunflower oil represents the mean food intake for adults in the Netherlands for a cooked meal regarding macronutrients and caloric composition and is based on the third Dutch National Food Consumption Survey from 1998 (Herman *et al.*, 2005). Immediately, 12 ml of stimulated gastric juice (pH 1.30 ± 0.02) is added and the mixture is rotated head-over-heels (55 rpm) for 2 h. The pH of the gastric fluid is determined, and the allowed interval is 2.5 ± 0.5. Subsequently, 12 ml stimulated duodenal juice (pH 8.1 ± 0.2), 6 ml stimulated bile (pH 8.2 ± 0.2), and 2 ml sodium bicarbonate (84.7 g/l) are added simultaneously. The mixture is rotated for another 2 h and the pH of the chyme was determined, with the allowed pH-interval 6.5 ± 0.5. Separation of chyme and pellet was obtained by centrifugation at 3000 g for 5 minutes. The whole process is performed at 37 ± 2 °C. Samples can be taken from the stomach and intestinal phase to obtain information on the bioaccessibility of the contaminant.

Note that the solid-to-liquid ratio for the digestion model for fasted and fed conditions are similar. For fasted conditions 0.06 and 0.6 g soil per digestion tube result in a solid-to-liquid ratio of 1(g soil):958 (l digestion fluid) and 1:96, respectively. For fed conditions 0.04 and 0.4 g soil per digestion tube result in a solid-to-liquid ratio of 1:1063 and 1:106, respectively. The rationale for these solid-to-fluid ratios is described in section 7.3.

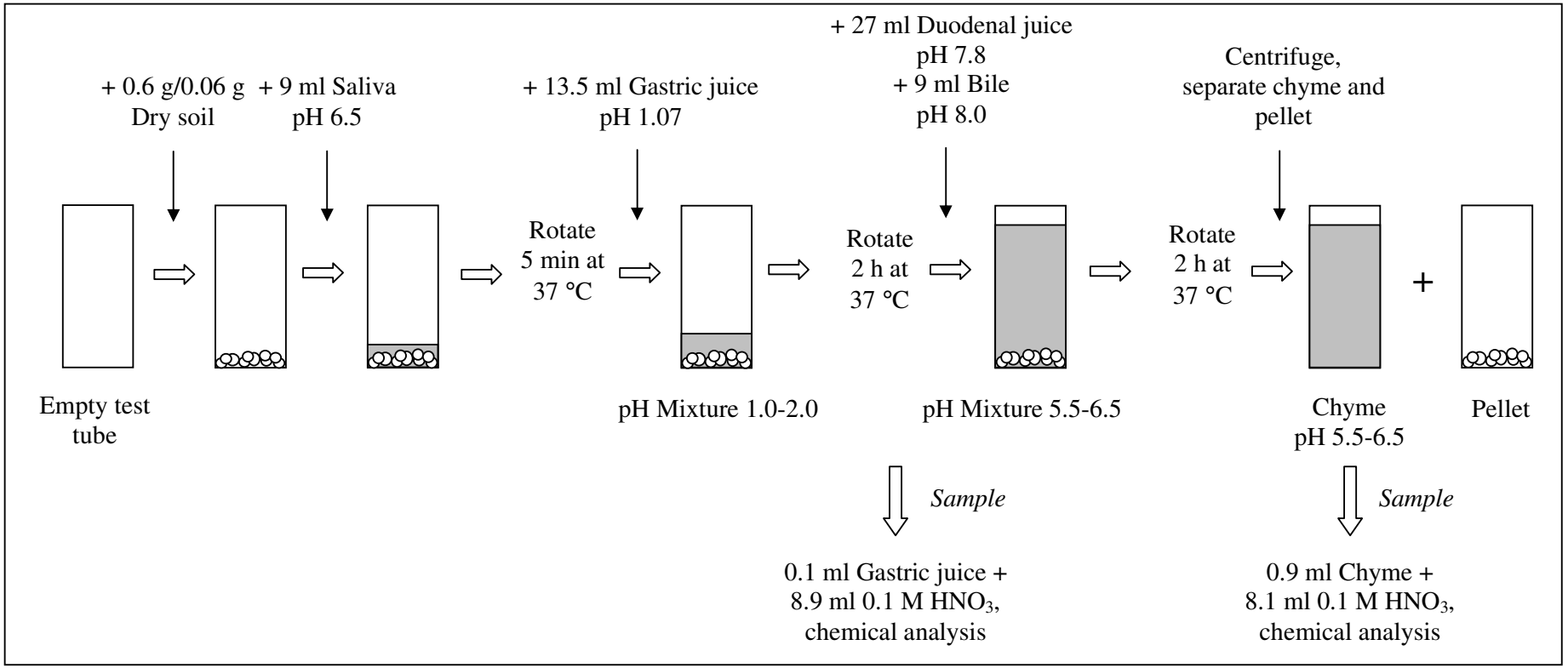

Figure 3. Schematic representation of the in vitro digestion procedure simulating fasted conditions

3.3 Chemical analysis

Although the RIVM *in vitro* digestion model can be used to investigate the bioaccessibility of all kinds of contaminants from soil, the present report focuses on lead, and thus only the analysis of lead is described.

For determining the lead concentration in chyme, 0.9 ml chyme is diluted tenfold with 8.1 ml HNO_3 (0.1 M). For determination of the lead concentration in gastric juice, 0.1 ml of gastric juice (stomach compartment) is diluted with 8.9 ml HNO_3 (0.1 M). Subsequently, lead is analyzed by means of Inductively Coupled Plasma/Mass Spectrometry (ICP-MS) (Perkin Elmer, Elan 6000).
If it was necessary to destruct pellets, the pellets of the *in vitro* digestion model are destructed by *aqua regia*. To destruct soil and pellet samples, demineralized water was added to 0.4-1.0 g soil or to the entire pellet, until a weight of 3 g was reached. Subsequently, 7 ml *aqua regia* was added, consisting of 1 part of HNO_3, 3 parts HCl, and 1 part demineralized water. This mixture was destructed in microwave pressure vessels (G-ACV-100) in a microwave (CEM MDS 2000). Finally, 0.9 ml of the destructed mixture was added to 8.1 ml HNO_3 (0.1 M), and analyzed by Inductively Coupled Plasma/Mass Spectrometry (ICPMS) (Perkin Elmer, Elan 6000).

3.4 Calculations

Equation 7 presents the calculation of bioaccessibility values. For each digestion tube:

$$\text{Bioaccessibility } (\%) = \frac{\text{amount of compound in chyme}}{\text{total amount of compound in soil}} \times 100\% \qquad (7)$$

The amount of contaminant in the chyme is determined from the concentration of contaminant in the chyme minus the amount of contaminant in the procedural blank, i.e. the digestion tube without soil that ran through all digestion steps. The procedural blank was in virtually all cases below the detection limit. The detection limit of lead in intestinal juice decreased from 40 µg/l to 5 µg/l in the course of the years in which this project was carried out. The limit of quantification of lead in soil and pellet was 2 mg/kg dry matter.

3.5 Mass balance

The amount of contaminant in the pellet can also be analysed. In that case, a mass balance can be made, i.e. the amount of the contaminant in the chyme and pellet should equal the amount of contaminant in the soil before the start of the digestion. This mass balance can be used to evaluate the quality of the experiments.

3.6 Conclusions

The present chapter describes the development and procedure of the RIVM *in vitro* digestion model, both for fasted and fed conditions. Also the calculation of bioaccessibility is addressed.

4. Other models to estimate oral bioavailability and bioaccessibility

In this chapter an overview of some relevant models to estimate oral bioavailability is given. The models are evaluated with regard to the issues that are considered important for a tool that should be able to give an estimate of a relative bioavailability factor. These issues are that the model should 1) avoid the use experimental animals, 2) be simple, 3) be cheap, 4) be fast, 5) be physiologically based, 6) be reliable, 7) be robust, 8) have a clear relationship to oral bioavailability.

4.1 Swine model

Juvenile swine are used as a model for young children to estimate the degree to which lead is bioavailable. A biological response is determined, for example area under the blood lead concentration-time curve, bone lead concentrations, terminal liver lead concentration, or terminal kidney lead concentration. This biological response is determined as a function of lead in soil and as a function of an orally administered soluble lead salt. In the USA, the obtained relative bioavailability can be used in risk assessment, and can be more or less than EPA's default relative bioavailability of soil versus water and food of 60% (Casteel *et al.*, 1997; US-EPA, 2002; US-EPA, 2004; US-EPA, 1999).
The juvenile swine model is expected to give reliable bioavailability values, as the swine's gastrointestinal tract is similar to the gastrointestinal tract of man. Major disadvantages of such a study are the need of test animals, the high costs (approximately 25000 dollar per soil sample), and amount of time needed for the experiments, see Table 1. Furthermore, there are limited possibilities to simulate various exposure scenarios.

4.2 Relative Bioavailability Leaching Procedure

An *in vitro* test in the USA is called the Relative Bioavailability Leaching Procedure (RBLP), which was developed by John Drexler (University of Colorado) (see also http:/www.colorado.edu/geolsci/legs/indexa.html) (Drexler *et al.*, In press; Ruby *et al.*, 1992). This model consists of a simulated stomach extraction only, i.e. intestinal conditions are not simulated, for the contaminants lead and arsenic. For the extraction 1.0 g of soil is added to 100 ml of a glycine buffered solution (0.4 M). The pH is set at 1.5 and the temperature at 37 °C. The mixture is rotated head-over-heels for 1 h. A sample is taken after filtration over a 0.45 µm disk filter (http:/www.colorado.edu/geolsci/legs/indexa.html).

For different contaminants, the model is adapted to make the best correlation to *in vivo* data. Hence, the pH in the stomach and the inclusion of the intestinal phase are contaminant dependent. This results in an empirical model rather than solely based on human physiology. Originally this *in vitro* model had both stomach and intestinal phases, but the model was simplified to a stomach extraction only for lead because the bioaccessibility results were comparable, regardless of whether a stomach and/or intestinal phase were used. The Relative Bioavailability Leaching Procedure is simple, cheap and reproducible, but only a few aspects of the human gastrointestinal tract are simulated, see Table 1.
Between institutes some slight differences exist. A slightly different model that has been developed in the USA is the Physiologically Based Extraction Test (PBET), by Ruby *et al.* (1993). As a result, it is unclear in which situations the model can be used.

4.3 TNO Gastrointestinal Model (TIM)

The TIM-model is a dynamic model that simulates the transit through the gastro-intestinal tract, the gastric and intestinal pH profiles, and the secretion of digestive juice over time (Minekus *et al.*, 1995).
Experiments with soil are generally performed while reproducing conditions that occur during digestion of a semi-liquid meal. To that end, ten grams of dry soil are introduced into 50 ml of artificial saliva of pH 5. After 5 minutes the mixture is added into 250 ml of artificial gastric content and transferred to the gastric compartment. Initially, the gastric pH is 5, afterwards it is controlled at pH 3.5, 2.5 and 2 for 30, 60 and 90 minutes, respectively. Subsequently, the soil and juices are gradually transferred to the intestinal compartments, representing the duodenum (pH 6.5), the jejunum (pH 6.8) and the ileum (pH 7.2). The gastric and duodenal secretions are set to 0.5 and 1 ml/minute, respectively. The total digestion time is 6 h. The chyme is mixed and transported by peristaltic movements. Dialysis membranes (Hospal, Molecular weight cutoff 5–10 kD) are used to remove bioaccessible contaminants, digestive metabolites and water from the chyme based on passive diffusion. Freely dissolved contaminants and small complexes can diffuse across the membranes. This process is efficient due to large quantities of dialysate (10 ml per minute) that are used to maintain the concentration gradient between chyme and dialysate. Hence, the contaminant fraction that is measured in the dialysate reflects the bioaccessible fraction.
This model has been validated by comparing the dissolution profile of drugs *in vivo* and *in vitro*, although little information on the validation is available in scientific literature.
The model is developed for commercial use and thus not freely available. Advantages of the TIM model are that no animals are used, and human physiology is simulated in detail. As a consequence, the model is relatively expensive, and time consuming, see Table 1. Before application in risk assessment, the relationship between bioaccessibility determined by the TIM-model and relative oral bioavailability should be established.

4.4 Unified BARGE method

In the spring of 2005 it was agreed upon by the members of the BioAccessibility Research Group Europe (BARGE) to develop one unified BARGE method. Up till that moment the members of the BARGE were comparing their different *in vitro* digestion models (Oomen *et al.*, 2002). RIVM was one of the founders of BARGE in 2000 and has been an active member since. It was decided to use the RIVM *in vitro* digestion model as a basis, and make a few adaptations. The composition of the digestive juices and the pH values are similar to the juices of the RIVM. The concentration of $NaHCO_3$ in the duodenal juice will be increased in the unified BARGE method to increase the pH in the intestinal phase slightly. Other changes are that the gastric phase will take 1 h instead of 2 h, and the intestinal phase 3 h or 4 h instead of 2 h. The pH in the stomach and intestinal phase should be within a certain range, and if not, the pH should be adjusted. An interlaboratory study will be performed with the unified BARGE method in 2006 to investigate whether the different institutes obtain the same outcome. This study will be performed with arsenic, cadmium, and lead contaminated soils of which *in vivo* bioavailability data for swine are available from the USA, so that also information on the relationship to oral bioavailability will be obtained.

The unified BARGE method is a major step forward to harmonisation of the use of bioavailability and bioaccessibility in human risk assessment of contaminated soils in Europe. The countries presently involved in BARGE are the UK, Belgium, Denmark, France, Canada, and the Netherlands. Germany might join the BARGE in the near future. If the outcome of the interlaboratory study is satisfactory, the unified BARGE method is likely to be incorporated in ISO-standards.

Table 1: Different models for estimation of a relative bioavailability factor are judged with a mark between 1 and 5, with higher numbers for better performance.

Model	Test animals	Simple	Cheap	Fast	Physiologically just	Reliable	Robust	Relationship to bioavailability
Swine model	Yes	1	1	1	5	5	4	5
RBLP	No	5	5	5	2	3	4	3
TIM	No	2	3	3	4	4	?*	?*
BARGE	No	3	4	4	3	?**	?**	?**
RIVM	No	3	4	4	3	4	4	4

* Little information available, probably good relationship to bioavailability.

** No data available yet.

4.5 Other models

A broad range of *in vitro* digestion models exist that have been applied in pharmaceutical research, food research, and for exposure assessment of consumer products, all having their own scope. These models vary from very simple chemically based to very sophisticated, for example including intestinal bacteria and gradual transfer from one compartment (e.g. stomach) to the next (e.g. intestine).

In order to investigate the effect of different *in vitro* digestion models on the bioaccessibility, the bioaccessibility of arsenic, cadmium and lead from 3 different soils was determined with five different models within the BARGE group. This resulted in a wide range of bioaccessibility values. The main differences in test results of bioaccessibility could be explained on the basis of the applied gastric pH. High bioaccessibility values were typically observed for a simple gastric method, which measured bioaccessibility in the gastric compartment at low pHs of 1.5. Other models that also applied a low gastric pH, and included intestinal conditions, produced lower bioaccessibility values. The lowest bioaccessibility values were observed for a gastrointestinal method which employed a high gastric pH of 4.0. For further details on the comparison between these five different models we refer to Oomen *et al.* (2002).

4.6 Conclusions

Some relevant models to estimate oral bioavailability are discussed. The swine model, which is an *in vivo* study, has as advantages that it is by definition physiologically correct, it is reliable and robust. On the other hand, the swine model uses test animals, is expensive and requires highly qualified personnel and equipment.
The other models are *in vitro* models, which have as a common advantage that no test animals are used. The *in vitro* models show a wide variety in the extent of simulation of physiological conditions, and connected a variety in simplicity, costs, and speed. For some *in vitro* digestion models a relationship with *in vivo* bioavailability has been derived.

5. Validation of the RIVM *in vitro* digestion model to *in vivo* data for lead

Obviously, the *in vitro* digestion model must be validated to the *in vivo* situation before the *in vitro* digestion model is reliable for use in risk assessment. The results of the *in vitro* digestion model are bioaccessibility values. Ideally, validation is performed by comparing ***in vitro bioaccessibility*** data to ***in vivo bioaccessibility*** data. However, *in vivo* bioaccessibility data are not available, as this cannot be measured. Instead, *in vivo* ***bioavailability*** data are compared to *in vitro* bioaccessibility data.
Validation of the *in vitro* digestion model is difficult because few *in vivo* data on bioavailability of lead from soil are available. *In vivo* studies were not possible in the present research due to the limited budget. Yet, by means of international contacts we were kindly supplied with several soils of which *in vivo* bioavailability information was available. In this manner, we could compare *in vitro* bioaccessibility data to *in vivo* bioavailability data of both humans (1 soil, fed and fasted conditions) and swine (10 soils) for lead. These results are discussed in section 5.2 and 5.3, respectively. This leads to a conclusion regarding the validation status of the *in vitro* digestion model of RIVM for lead in soil. In addition, research is addressed in which *in vitro* bioaccessibility and *in vivo* bioavailability values for other contaminants than lead is compared (chapter 6). The latter gives an idea of the generality of the applicability of the *in vitro* digestion model.

Besides the validation of the *in vitro* digestion model to the *in vivo* data, validation also involves “experimental” validation, i.e. within- and between-day variability, intra- and interlaboratory variability, reproducibility etc. The within-day and between-day variability has been studied in the past (Sips *et al.*, 2001). At that time, the within-day variation typically ranged between 5 and 20%, and the between-day variation between 11 and 79%. These data suggest that the variability is not very good. However, in recent years much effort has been put in better standardizing the *in vitro* digestion procedure, which is also apparent from Figure 9 in this report, which shows the bioaccessibility of lead from soil at many different spiked contamination levels for 4 different soil types. In these studies the standard deviation of the bioaccessibility ranged between 1.3 and 10.9%, including the variability introduced by spiking at different contaminant levels. These data suggest that the reproducibility of the *in vitro* digestion procedure is satisfactory.

5.1 Preconditions of *in vivo* determined bioavailability data

The *in vivo* bioavailability data that are used to validate the RIVM *in vitro* digestion model should be good and suitable for *vitro-vivo* comparison. The best data can obviously be

obtained from human studies. A human study was only performed for one soil (Maddaloni *et al.*, 1998), which is also used for the present *vitro-vivo* comparison. However, when human data are not available, the animal species used in the bioavailability studies should have a physiology similar to humans. **Swine** have several gastric features in common with humans, especially for the fasted state (De Zwart *et al.*, 1999). For example, both swine and human possess a simple stomach consisting of only one compartment. Also the gastric pH for fasted conditions is similar, on average 1.7 for humans and 1.6-1.8 for swine. In contrast to humans and swine, rodents (mouse, rat, rabbit) are continuous feeders, which means that in a healthy animal the stomach is never empty. This enables the maintenance of gastric floral growth required by rodents for digestion of cellulose (De Zwart *et al.*, 1999). Therefore, **swine are considered a suitable species for *vitro-vivo* comparison, whereas rat, mice and rabbit are not.** In addition, the swine studies should have been performed for **fasted conditions.** Data on bioavailability of lead from soil for other animal species are not available.

5.2 Comparison to human bioavailability data of lead-contaminated soil

Two oral bioavailability values were determined in a volunteer study on oral bioavailability of lead from ingested soil: oral bioavailability of lead from Bunker Hill soil for fasted and for fed conditions (Maddaloni *et al.*, 1998). The Bunker Hill soil used in the volunteer study was kindly donated to the BARGE, including the RIVM, by Mark Maddaloni of the US-EPA. Within the RIVM, *in vitro* bioaccessibility values for fasted conditions were obtained by using the *in vitro* digestion model for fasted conditions as developed in the present project (see subsection 3.2.1). Bioaccessibility for fed conditions were obtained with the *in vitro* digestion model for fed conditions developed by the project "*in vitro* digestion model food/toy", see subsection 3.2.2 (Versantvoort *et al.*, 2004; Versantvoort *et al.*, 2005).
For validation of the *in vitro* digestion model, oral bioavailability of lead for fed conditions (F_{fed}) and fasted conditions (F_{fasted}) as obtained from the *in vivo* study should be compared to the *in vitro* bioaccessibility of lead from soil for fed conditions ($F_{B,fed}$) and fasted conditions ($F_{B,fasted}$), respectively.

To that end, knowledge on the absorption of lead for fed ($F_{A,fed}$) and fasted ($F_{A,fasted}$) conditions, and metabolism of lead for fed ($F_{H,fed}$) and fasted ($F_{H,fasted}$) conditions is required. As lead is not metabolised in humans (Diamond *et al.*, 1997), $F_{H,fed}$ and $F_{H,fasted}$ both equal 1. Absorption of lead depends on the physiological state, i.e. lead absorption is different for fasted conditions than for fed conditions. The reason is probably that lead competes with calcium for absorption, whereas also interaction of lead with iron, phosphate and vitamin D may occur (Mushak, 1991; Heard *et al.*, 1982; Diamond *et al.*, 1997; James *et al.*, 1985; Blake *et al.*, 1983). As food contains those modulating contaminants, $F_{A,fed}$ does not equal

$F_{A,fasted}$. Hence, knowledge on the absorption of lead for both physiological conditions is required for comparison of *in vivo* bioavailability data with *in vitro* bioaccessibility data.

$$F_{fed} = F_{B,fed} \times F_{A,fed} \times F_{H,fed} \quad (8)$$

$$F_{fasted} = F_{B,fasted} \times F_{A,fasted} \times F_{H,fasted} \quad (9)$$

Below, *in vivo* bioavailability of lead from Bunker Hill soil is compared with *in vitro* bioaccessibility for fasted conditions and fed conditions.

5.2.1 Fasted conditions

For fasted conditions in the study by Maddaloni *et al.* (1998), oral bioavailability of lead was 26% after ingestion of Bunker Hill soil, i.e. F_{fasted}=0.26. This value should be compared to the bioaccessibility of lead from Bunker Hill soil, but first figures for the absorption of bioaccessible lead should be derived. Subsequently, the bioaccessibility of lead from Bunker Hill soil is compared to the bioavailability of lead from Bunker Hill soil determined in humans.

In this paragraph, a range for absorption of bioaccessible lead is derived. The bioaccessibility of well-soluble lead acetate for fasted conditions was determined in the *in vitro* digestion model and was found to be 66%, i.e. $F_{B,fasted}$ = 0.66. The bioavailable fraction of lead from an aqueous solution for fasted conditions as reported in literature ranges between 0.3 and 0.7, i.e. F_{fasted} = 0.3-0.7 (James *et al.*, 1985; Heard *et al.*, 1983; Heard *et al.*, 1982; Rabinowitz *et al.*, 1980). Hence, an absorption-factor can be deduced:

$$F_{fasted} = F_{B,Pbacetate} \times F_{A,fasted} \quad (10)$$

$$F_{A,fasted} = \frac{(\text{range } 0.3\text{-}0.7)}{0.66} = (\text{range } 0.45\text{-}1.06) \quad (11)$$

As absorption of lead from water can never exceed 100%, the range of $F_{A,fasted}$ is 0.45-1.0. This absorption factor is partially method-defined, as the method of separating chyme from digested soil influences the bioaccessibility. Furthermore, it should be noted that in principle oral bioavailability should never exceed bioaccessibility, as bioaccessibility is a sub-process of oral bioavailability.

The bioaccessibility of lead from Bunker Hill soil determined with the RIVM *in vitro* digestion model was 45.4 ± 4.0 with 0.06 g of soil per digestion tube (n=7, 3 different experiments), and 29.6 ± 5.1 with 0.6 g of soil per digestion tube (n=6, 3 different experiments).

By combining *in vivo* bioavailability of lead (0.26) with the bioaccessible fraction of lead from Bunker Hill soil determined by the RIVM in the *in vitro* digestion model (0.30-0.45), and the range of the fraction absorbed for fasted conditions (0.45-1.0), the following equation can be made:

$$F_{fasted} = 0.26 = (\text{range } 0.30 - 0.45) \times (\text{range } 0.45 - 1.0) = (\text{range } 0.14 - 0.45) \qquad (12)$$

Although the range of 0.45-1.0 for absorption of bioaccessible lead is large, the *in vivo* bioavailability data for fasted conditions (0.26) is in agreement with the calculated oral bioavailability (0.14-0.45) based on *in vitro* bioaccessibility data with the RIVM fasted model (0.30 – 0.45) and data on the absorption of lead (0.45-1.0).
Note that lead-complexes in the chyme that are not absorbed *in vivo* are taken into account by the absorption factor.

5.2.2 Fed conditions

For comparison of the *in vivo* bioavailability of lead from Bunker Hill soil with *in vitro* bioaccessibility values, first an estimate of the absorption of lead for fed conditions ($F_{A,fed}$) should be made. The bioavailable fraction of lead from food is low, between 0.03 and 0.09 (James *et al.*, 1985; IPCS, 1995; Heard *et al.*, 1983; Heard *et al.*, 1982; Rabinowitz *et al.*, 1980). The bioaccessible fraction of lead from food matrices as determined with the RIVM *in vitro* digestion model ranged between 50 and 80%. Hence, the $F_{A,fed}$ is estimated to be:

$$F_{fed} = F_{B,fed} \times F_{A,fed} \qquad (13)$$

$$(\text{range } 0.03\text{-}0.09) = (\text{range } 0.5\text{-}0.8) \times F_{A,fed} \qquad (14)$$

$$F_{A,fed} = \frac{(\text{range } 0.03\text{-}0.09)}{(\text{range } 0.5\text{-}0.8)} = (\text{range } 0.04\text{-}0.18) \qquad (15)$$

This indicates that for fed conditions 4-18% of the bioaccessible lead is transported across the intestinal wall.

The bioavailable fraction of lead from Bunker Hill soil for the fed state in the Maddaloni study was 0.025 (Maddaloni *et al.*, 1998). The bioaccessible fraction of lead from Bunker Hill soil after *in vitro* digestion simulating fed conditions was on average 28.9 ± 6.9 (n=26; obtained for digestions with increasing amounts of food, e.g. breakfast, and independent on the two amount of soil, 0.04, 0.01, or 0.4 g). For simulation of fed conditions, the same breakfast was made as was used by Maddaloni *et al.* (1998). These figures are compared with the estimated fraction of lead absorbed in presence of food (0.04-0.18):

$$F_{fed} = F_{B,fed} \times F_{A,fed} \quad (16)$$

$$0.025 = 0.29 \times (\text{range } 0.04\text{-}0.18) = (\text{range } 0.012\text{-}0.052) \quad (17)$$

Hence, although the range is wide, the *in vivo* bioavailability of lead from Bunker Hill soil (0.025 or 2.5%) is in agreement with the calculated oral bioavailability (0.012-0.052 or 1.2-5.2%) based on *in vitro* bioaccessibility (0.29 or 29%) and absorption data of bioaccessible lead (0.04-0.18 or 4-18%).

5.3 Comparison to swine bioavailability data of lead-contaminated soil

Soils historically contaminated with lead were kindly provided by Christian Grøn (DHI, Denmark), who in turn obtained the soils from Prof. Dr. Stan Casteel (University of Missouri, Columbia, USA), who had performed the *in vivo* studies in juvenile swine. The soils listed in Table 2 were obtained.

Table 2: Soils with known relative oral lead bioavailability determined in a juvenile swine study (US-EPA, 2004).

Soil	Lead concentration (mg/kg)	Relative *in vivo* bioavailability in juvenile swine (%)*	LB	UB
Jasper LL yard soil	4050	90	63	120
Murray smelter slag	11500	40	23	64
Jasper HL mill soil	6940	82	51	114
Midvale slag	8170	14	7	24
Butte soil	8530	14	6	23
California Gulch Fe/Mn PbO	4320	105	57	156
Murray smelter soil	3200	51	29	79
NIST paint + soil	8350	72	44	98
Galena enriched soil	11200	1	0	3
California Gulch Oregon Gulch Tailings	1270	6	-1	15

LB = 5% Lower Confidence Bound

HB = 95% Upper Confidence Bound

* Bioavailability of soil relative to the bioavailability of soluble lead-acetate (see 5.3.1). Of some of the listed soils bioavailability data have been published by Ruby et al. (1999) and Schroder et al. (2004). The relative bioavailability values as reported by the US-EPA were adopted as these were re-evaluated and more extensive bioavailability studies were performed (US-EPA, 2004).

5.3.1 *In vivo* bioavailability study

The soils listed in Table 2 were tested on oral bioavailability in an juvenile swine dosing study (Schroder *et al.*, 2004; Ruby *et al.*, 1999; US-EPA, 2004). Five male swine (5-6 weeks old, 10-12 kg) were used per dosing group. The swine were dosed twice daily for 15 days after an overnight fast and after a 4-h fast in the afternoon. In this manner, a fasted or semi-fasted state was simulated. Half of the soil dose was administered in the morning and the other half in the afternoon. Soil doses were placed in the centre of a 5-10 g doughball of moistened diet.
Blood and other tissues (kidney, liver and bone) were analysed for lead, and used as a measure of lead bioavailability. A relative bioavailability value was obtained by comparing the measure of lead bioavailability from soil by the measure of lead bioavailability of the well-soluble lead acetate.
All soil samples were re-evaluated by the US-EPA, i.e. more extensive bioavailability studies were performed (US-EPA, 2004). To that end, three different doses were administered to juvenile swines. Blood samples were taken multiple times during the course of the experiment, so that an AUC (Area Under the Curve, i.e. blood concentration integrated over time) value could be derived. For liver, kidney, and bone the measure of response was the concentration of lead in these tissues on day 15. Most dose-response curves for liver, kidney, and bone lead were well described by a linear model, and most blood lead AUC data sets were well described by an exponential model. Dose-response curves were also obtained from the response after 3 doses of the well-soluble lead acetate. Relative bioavailability values were obtained by the ratios of the doses of soil and lead acetate that produced equal responses, i.e. equal AUC, liver, kidney, or bone lead. Uncertainty bounds, i.e. 5% lower confidence bound and 95% upper confidence bound, were adopted from the US-EPA (US-EPA, 2004). For presentation in Figure 4, the standard deviation of the oral bioavailability of soils was calculated as half the interval between the point estimate and the 95% confidence bound.

5.3.2 *In vitro* digestion model

The soils listed in Table 2 were digested in the RIVM fasted *in vitro* digestion model. In addition, the bioaccessibility of well-soluble lead acetate was determined (stomach 91.4 ± 2.6%, intestine 66.2 ± 1.5%), and used to calculate the relative bioaccessibility of the soils in respectively the stomach and intestinal compartment. The relative *in vitro* bioaccessibility and the relative *in vivo* bioavailability data derived from the *in vivo* juvenile swine study are correlated in Figure 4.

As can be seen in Figure 4, the correlation between relative *in vitro* bioaccessibility and relative *in vivo* bioavailability data is good when data points are excluded for which the pH in the stomach compartment of the *in vitro* digestion model were above pH 2 (open squares). The pH of the excluded data point for the experimental series with 0.06 g per digestion tube was 2.6, and for excluded data points in the series with 0.6 g per digestion tube between

pH 4.3 and 4.95. Such high pH values are not expected in the *in vivo* situation as additional acid secretion would lower the pH again. Hence, these data points can be excluded from a physiological point of view. As a consequence, most usable data points (9) were obtained with 0.06 g soil per digestion tube. For digestions with 0.6 g soil per digestion tube 6 soils could be used for the correlation between bioaccessibility and bioavailability. The correlation of the remaining data is good, with r^2-values ranging between 0.66 and 0.95.
The absolute and relative bioaccessibility values of these soils are listed in Table 3 for 0.06 g soil per digestion tube, and Table 4 for 0.6 g soil per digestion tube.

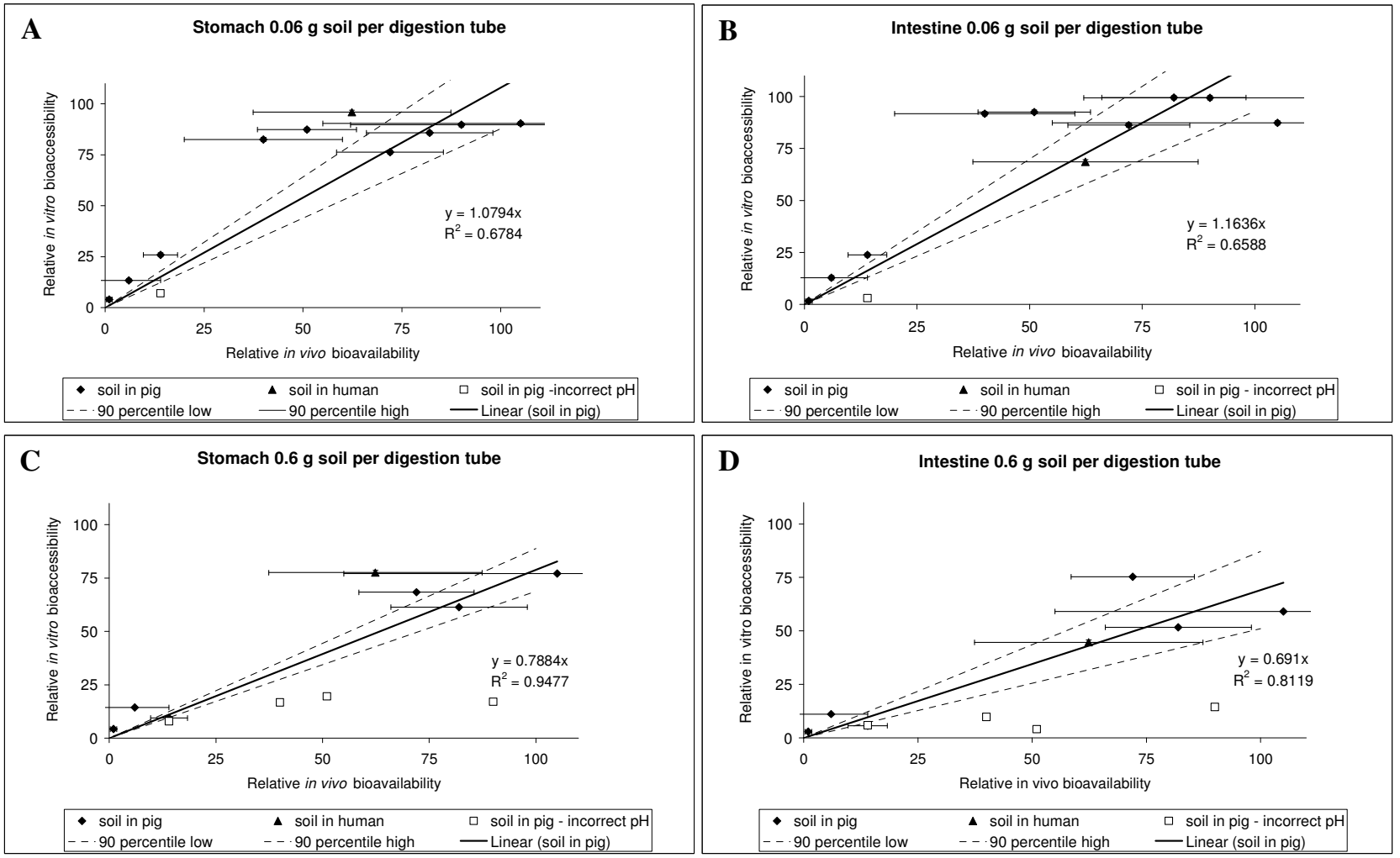

*Figure 4. Correlation of **lead** between **relative in vivo bioavailability** data obtained with juvenile swine studies (US-EPA, 2004; Schroder et al., 2004; Ruby et al., 1999) and **relative in vitro bioaccessibility** data obtained with the RIVM in vitro digestion model. The data indicated with the*

open squares are not included in the correlation as they displayed very high, not-physiological gastric pH values during the in vitro digestion (see text). The triangles represent the data of lead bioavailability from Bunker Hill soil, which was determined in adult volunteers (see section 5.2, Bunker Hill data not included in correlation). The full line indicates a linear fit to the data with a forced intercept through zero, i.e. if a compound is not bioaccessible it should not be bioavailable either. The dotted lines indicate the associated 90% interval of the fitted lines.

*Table 3. The values of the relative oral bioavailability of lead from soils determined in the juvenile swine study (see Table 2), and the bioaccessibility values for in vitro digestions with **0.06 g soil per digestion tube**. Both the absolute and relative bioaccessibility values for stomach and intestine are listed.*

		Bioaccessibility stomach		Bioaccessibility intestine	
Soil	Relative bioavailability (%)	Absolute (%)	Relative (%)	Absolute (%)	Relative (%)
Jasper LL yard	90 ± 28	82.0 ± 7.8	89.7 ± 8.9	65.7 ± 9.3	99.3 ± 14.3
Murray smelter slag	40 ± 20	75.4 ± 1.2	82.5 ± 2.7	60.7 ± 1.8	91.7 ± 3.4
Jasper HL mill	82 ± 16	78.4 ± 0.4	85.7 ± 2.5	65.8 ± 1.1	99.4 ± 2.8
Midvale slag	14 ± 4	6.4 ± 0.4	7.0 ± 0.5	2.1 ± 0.0	3.1 ± 0.1
Butte soil	14 ± 4	23.7 ± 0.4	25.9 ± 0.9	15.8 ± 0.3	23.8 ± 0.7
California Gulch Fe/Mn PbO	105 ± 50	82.6 ± 0.1	90.4 ± 2.6	57.8 ± 1.8	87.3 ± 3.4
Murray smelter soil	51 ± 13	79.8 ± 0.6	87.3 ± 2.6	61.3 ± 1.3	92.5 ± 2.9
NIST paint + soil	72 ± 14	69.7 ± 1.0	76.3 ± 2.4	57.1 ± 0.8	86.2 ± 2.3
Galena enriched soil	1 ± 1	3.7 ± 0.6	4.0 ± 0.7	1.1 ± 0.1	1.7 ± 0.2
California Gulch Oregon Gulch Tailings	6 ± 8	12.2 ± 0.3	13.3 ± 0.5	8.5 ± 0.2	12.9 ± 0.4
Bunker Hill*	62 ± 25	87.6 ± 8.4	95.8 ± 9.6	45.4 ± 4.0	68.6 ± 6.2

The shaded lines indicate that the corresponding soil resulted in a pH in the stomach compartment of the *in vitro* digestion model above the allowed range (1 < pH < 2). Consequently, the results of these soils should not be used in the correlation between *in vivo* bioavailability and *in vitro* bioaccessibility.
* Bioavailability of lead from Bunker Hill soil was determined in humans (Maddaloni *et al.*, 1998) and recalculated to a relative bioavailability factor, see section 5.3.3.

*Table 4. The values of the relative oral bioavailability of lead from soils determined in the juvenile swine study (see Table 2), and the bioaccessibility values for in vitro digestions with **0.6 g soil per digestion tube**. Both the absolute and relative bioaccessibility values for stomach and intestine are listed.*

		Bioaccessibility stomach		Bioaccessibility intestine	
Soil	Relative bioavailability (%)	Absolute (%)	Relative (%)	Absolute (%)	Relative (%)
Jasper LL yard soil	90 ± 28	15.6	17.1	9.6	14.4
Murray smelter slag	40 ± 20	15.4	16.8	6.5	9.8
Jasper HL mill soil	82 ± 16	56.1	61.3	34.2	51.7
Midvale slag	14 ± 4	7.3	8.0	3.9	5.9
Butte soil	14 ± 4	8.6	9.4	3.8	5.7
California Gulch Fe/Mn PbO	105 ± 50	70.4	77.0	39.1	59.1
Murray smelter soil	51 ± 13	17.9	19.6	2.7	4.1
NIST paint + soil	72 ± 14	62.5	68.4	49.8	75.3
Galena enriched soil	1 ± 1	3.9	4.3	1.9	2.9
California Gulch Oregon Gulch Tailings	6 ± 8	13.2	14.4	7.4	11.1
Bunker Hill*	62 ± 25	70.9 ± 0.9	77.6 ± 2.4	29.6 ± 5.1	44.7 ± 7.8

The shaded lines indicate that the corresponding soil resulted in a pH in the stomach compartment of the *in vitro* digestion model above the allowed range (1 < pH < 2). Consequently, the results of these soils should not be used in the correlation between *in vivo* bioavailability and *in vitro* bioaccessibility.
* Bioavailability of lead from Bunker Hill soil was determined in humans (Maddaloni *et al.*, 1998) and recalculated to a relative bioavailability factor, see subsection 5.3.3.

From a physiological point of view, it is expected that the slope of the relative bioaccessibility – relative bioavailability correlation in both the stomach and the intestinal compartment is "1", see equation 18. Precondition is that no re-delivery of the non-bioaccessible to the bioaccessible contaminant fraction occurs during transfer of the contaminant through the gastrointestinal tract.
Mathematically the reasoning is as follows. Absorption of bioaccessible lead from soil and lead acetate can be assumed to be equal as both occur for fasted conditions. Thus, $F_{A,soil}/F_{A,Pbacetate}$ is 1. In addition, $F_{H,soil}/F_{H,Pbacetate}$ is also 1 since lead is not metabolized.

$$\text{Slope}=\frac{\text{y-axis}}{\text{x-axis}}=\frac{Rel\ F_B}{Rel\ F}=\frac{\frac{F_{B,soil}}{F_{B,Pbacetate}}}{\frac{F_{B,soil}}{F_{B,Pbacetate}}\times\frac{F_{A,soil}}{F_{A,Pbacetate}}\times\frac{F_{H,soil}}{F_{H,Pbacetate}}}=\frac{\frac{F_{B,soil}}{F_{B,Pbacetate}}}{\frac{F_{B,soil}}{F_{B,Pbacetate}}\times 1\times 1}=1 \qquad (18)$$

As can be seen in Figure 4, the slope of the line was close to 1, especially in Figure A and B, in which case the *in vitro* digestion experiments were performed with 0.06 g soil per tube. Differences between the slope of the line observed between the series of digestion with 0.06 g and 0.6 g soil per digestion tube may be due to the differences in solid-to-liquid ratio, with perhaps saturation of lead in the digestive juice at low solid-to-liquid ratios, or because the pH is slightly lower in the digestive juices for digestion with 0.06 g of soil.

The correlation was better for the digestions with 0.6 g soil per tube (r^2 0.9477 in the stomach and 0.8119 in the intestine) was better than for the digestions with 0.06 g soil per tube (r^2 0.6784 in the stomach and 0.6588 in the intestine). This can be mostly attributed to the lower number of valid data-points in Figure 4C and D compared to Figure 4A and B.

The correlation between *in vivo* relative bioavailability and *in vitro* relative bioaccessibility in the stomach and intestinal compartments are similar. For further testing we recommend to determine bioaccessibility in the **intestinal compartment**, even though a test simulating the stomach only is simpler than the full *in vitro* gastrointestinal model. The full gastrointestinal model is in better agreement with human physiology, as lead absorption occurs primarily in the intestine. In this manner, the chance on correct bioaccessibility values is higher even for soil types that are different from the ones used in the *vitro-vivo* comparison.
In addition, **we recommend using 0.06 g of soil per digestion tube** for the time being. By using such a small amount of soil per digestion tube, the pH during *in vitro* digestion is usually not affected by the soil, so that in almost all cases the results can be used. The correlation is lower than for 0.6 g soil per digestion tube, but this can be attributed to the higher number of valid data points. In addition, the slope of the correlation is in better agreement with the expectation. Disadvantages of using such small amounts of soil are that 1) the aliquot of soil taken for bioaccessibility determination might not be representative for the entire soil, and 2) this may give rise to difficulties with the detection of lead in the digestive juices. An option would be to use larger amounts of soil (0.6 g per digestion tube)

and adjust the gastric pH if this appears to be outside the allowed range (1 < pH < 2). However, before this procedure can be applied the correlation with *in vivo* bioavailability data should be checked for soils after pH adjustment.
The satisfactory *vitro-vivo* correlation suggests that the *in vitro* digestion model is a suitable model to assess the bioavailability of lead from soil, and that additional delivery of non-bioaccessible lead to bioaccessible lead *in vivo* (see section 2.1) does not occur.

5.3.3 Comparison to swine bioavailability data to human bioavailability data

Besides data of the juvenile swine study also the relative *in vitro* bioaccessibility and relative *in vivo* bioavailability data obtained from an adult volunteer study with Bunker Hill soil (Maddaloni *et al.*, 1998) are included in Figure 4 (open triangles, Bunker Hill data are not included in the correlation as the correlation is based on bioavailability data from swine). Note that the oral bioavailability of lead from Bunker Hill had to be divided by the oral bioavailability of lead from lead acetate or another soluble lead form, in order to obtain a value for the relative bioavailability of lead from Bunker Hill soil. The bioavailability of soluble lead forms in human studies was found to range between 30 and 70% (James *et al.*, 1985). Hence, the relative oral bioavailability of lead from Bunker Hill soil was:

$$Rel\ F_{\text{Bunker Hill soil}} = \frac{F_{\text{Bunker Hill soil}}}{F_{\text{soluble lead form}}} = \frac{0.26}{(\text{range } 0.3\text{-}0.7)} = (\text{range } 0.37\text{-}0.87) \qquad (19)$$

Another difference that might affect the comparison between Bunker Hill soil oral bioavailability and the oral bioavailability data of the juvenile swine study is the age of the tested subject. Juvenile swine might absorb lead to a greater extent than human adults. Yet, the bioaccessibility and oral bioavailability data point of Bunker Hill fits reasonable well within the correlation obtained with the juvenile swine study.

5.3.4 Between-laboratory variability

The same and some additional soils as listed in Table 3 and 4 were used by Christian Grøn and co-workers (DHI, Denmark) who also compared the bioaccessibility of lead from these soils to the relative *in vivo* bioavailability data (Gron, 2005). Among others, they used the methodology of the RIVM *in vitro* digestion model, and found a poor correlation in the intestinal compartment and a relatively good correlation in the stomach compartment (Gron, 2005). In these studies 0.6 g of soil was used per digestion tube. Perhaps this or some other differences between the laboratories caused the poor correlation found for the intestinal compartment. However, also for other soil, e.g. Bunker Hill soil, differences in bioaccessibility were observed between DHI and RIVM. This stresses the need for more comprehensive between-laboratory testing in the future.

5.4 Other studies relating bioaccessibility to oral bioavailability of lead-contaminated soils

Experiments using several techniques to separate chyme from pellet can be used to clarify the relationship between bioaccessibility and oral bioavailability of lead from soil. This information was obtained in a study by the BARGE members on Bunker Hill soil. In the RIVM model the chyme is separated from the pellet by a centrifugation step at 3000 *g*. Hence, only large particles like soil particles are precipitated. The TIM model of TNO employs a continuous dialysis method, so that very small lead particles and freely dissolved lead are transported continuously across the dialysis membrane (Oomen *et al.*, 2002). TNO found bioaccessibility values that were relatively close to the oral bioavailability values for Bunker Hills. For the fasted situation a bioaccessibility of 33 ± 5% was determined by TNO, and for the fed situation values of 7 ± 2%, whereas for *in vivo* bioavailability values of respectively 26% and 2.5% were found. When RIVM applied ultrafiltration (Molecular Weight cutoff of 10 kD) to the centrifuged chyme samples after an *in vitro* digestion with Bunker Hill soil and breakfast, e.g. fed conditions, the bioaccessibility of lead decreased from 30.6 ± 7.5% (n=8, range 21-42%, with varying amounts of food, e.g. breakfast, and 2 different amounts of soil) to 3.1 ± 2.7% (n=8, range 0.4-6.6%, with varying amounts of food, e.g. breakfast, and 2 different amounts of soil). After filtration of the digestive juice samples over 0.45 µm, the bioaccessibility of the samples was in between the bioaccessibility obtained after centrifugation and ultrafiltration: on average 19.8 ± 5.7 (n=8, range 14-30%, with varying amounts of food, e.g. breakfast, and 2 different amounts of soil). Hence, after ultrafiltration the bioaccessibility values of RIVM closely match the *in vivo* bioavailability data and the bioaccessibility values of TNO. These data suggest that all freely dissolved lead and the very small lead particles are transported across the intestinal epithelium. It obviously also indicates that the method of separating digestive fluid from the digested soil in the *in vitro* digestion procedure affects the comparison and relationship between *in vitro* bioaccessibility and *in vivo* bioavailability substantially. As this study only concerns one soil, a firm conclusion about the method of separating chyme and pellet in relation to the oral bioavailability cannot be drawn. Further research on the method of separation is recommended.

5.5 Conclusions

The correlation between *in vitro* relative bioaccessibility determined by the RIVM *in vitro* digestion model and relative bioavailability of lead from soil determined in juvenile swine was satisfactory. For the experiments in which the intestinal bioaccessibility was determined from 0.06 g soil per digestion tube, the r^2-value of the correlation was 0.6588 (Figure 4B), whereas also the slope of the line was according expectations. For further testing we

recommend to determine bioaccessibility in the intestinal compartment, which is in agreement with physiology, as lead absorption occurs primarily in the intestine. In addition, we recommend using 0.06 g of soil per digestion tube for the time being. By using such a small amount of soil per digestion tube, the pH during *in vitro* digestion is usually not affected by the soil, so that in almost all cases the results can be used. However, disadvantages of using such small amounts of soil are that 1) the aliquot of soil taken for bioaccessibility determination may be less representative for the entire soil, and 2) this may give rise to difficulties with the detection of lead in the digestive juices. An option would be to use larger amounts of soil (0.6 g per digestion tube) and adjust the gastric pH if this appears to be outside the allowed range ($1 < pH < 2$). However, before this procedure can be applied the correlation with *in vivo* bioavailability data should be checked for soils after pH adjustment.

6. Validation of the RIVM *in vitro* digestion model to *in vivo* data for other compounds than lead

During the course of this project and in a related project on the bioavailability of compounds from food and toy matrices (V/320102), addition information on the relationship between bioaccessibility and oral bioavailability is obtained. This concerns 1) arsenic from soil, 2) cadmium from soil, 3) ochratoxin A and aflatoxin B from food, and 4) phthalate from PVC disks. The research on cadmium from soil was performed by Christian Grøn and co-workers (DHI, Denmark), who have implemented and validated the Relative Bioavailability Leaching Procedure (a variation of the PBET method, developed by John Drexler, University of Colorado) and the RIVM *in vitro* digestion model in their present research for the Danish EPA. The other compounds are studied at the RIVM. The research on the different compounds is addressed in more detail below.

6.1 Arsenic from soil

Soils historically contaminated with arsenic were kindly provided by Nick Basta (Ohio State University, USA). These soils were tested on oral bioavailability in an juvenile swine dosing trial (Rodriguez *et al.*, 2003). Five male swine of 10-12 kg were used per treatment group, and were dosed with 6.25 mg soil per kg body weight per day. One-half of the dose was administered 2 h before feeding in the morning and the remaining half given 2 h before the afternoon feeding, so that the soils were ingested in fasted conditions. Soil doses were placed in the centre of a 5-g portion diet material. Urine excretions were collected and analysed. In most animals, including swine, absorbed arsenic is excreted primarily in urine (IPCS (International Programme on Chemical Safety), 2001). Arsenic excretion in urine was found to be a linear function of the administered dose. Hence, the fraction of arsenic excreted in the urine is a measure of the absolute oral bioavailability. The relative bioavailability of arsenic is the absolute oral bioavailability of the studied soil divided by the absolute oral bioavailability of a reference material, in this case a highly soluble arsenic form ($Na_2AsO_4 \cdot 7H_2O$). The relative bioavailability can thus be obtained from the urinary arsenic excretion after soil ingestion divided by the urinary arsenic excretion of the soluble arsenic form ($Na_2AsO_4 \cdot 7H_2O$).

The soils were tested in the *in vitro* digestion model in order to obtain bioaccessibility values. Also the soluble arsenic $Na_2AsO_4 \cdot 7H_2O$ was tested in the digestion model so that corresponding relative bioaccessibility values could be calculated, by dividing the bioaccessibility of the soil by the bioaccessibility of the soluble arsenic $Na_2AsO_4 \cdot 7H_2O$, which was 91% in the stomach and 88% in the intestine. In this manner, the relative oral

bioavailability could be correlated to the relative bioaccessibility in order to validate the outcome in the *in vitro* digestion model.

Figures 5A to D show the correlation between the relative bioavailability and relative bioaccessibility of arsenic for various soils. The bioaccessibility in the stomach and intestinal compartment is plotted for **0.06 g** of soil per digestion tube in Figures 5A and 5B, respectively. The bioaccessibility in the stomach and intestinal compartment is plotted for **0.6 g** of soil per digestion tube in Figures 5C and 5D, respectively. As can be seen, the correlation is good for 0.06 g of soil per digestion tube, with an r^2-value of 0.908 in the stomach and 0.796 in the intestine. When 0.6 g of soil per digestion tube is used, the correlation in the stomach is good (r^2-value of 0.937), but not good in the intestine (r^2-value of 0.227). The low correlation can be ascribed to the high pH values for some soils in the stomach compartment (ranging from 2.6 to 4.9), whereas pH values between 1 and 2 in the stomach are considered acceptable. Apparently, the buffering capacity of the digestive juices is not high enough for these soils when 0.6 g per digestion tube is used. The data points for soils with high pH in the stomach compartment are indicated by the open squares, whereas data points with the acceptable pH range during *in vitro* digestion are represented by solid squares. The determination of a correlation in Figure 5C and D for soils with acceptable pH values during *in vitro* digestion is not possible as then only 3 data points would be left.

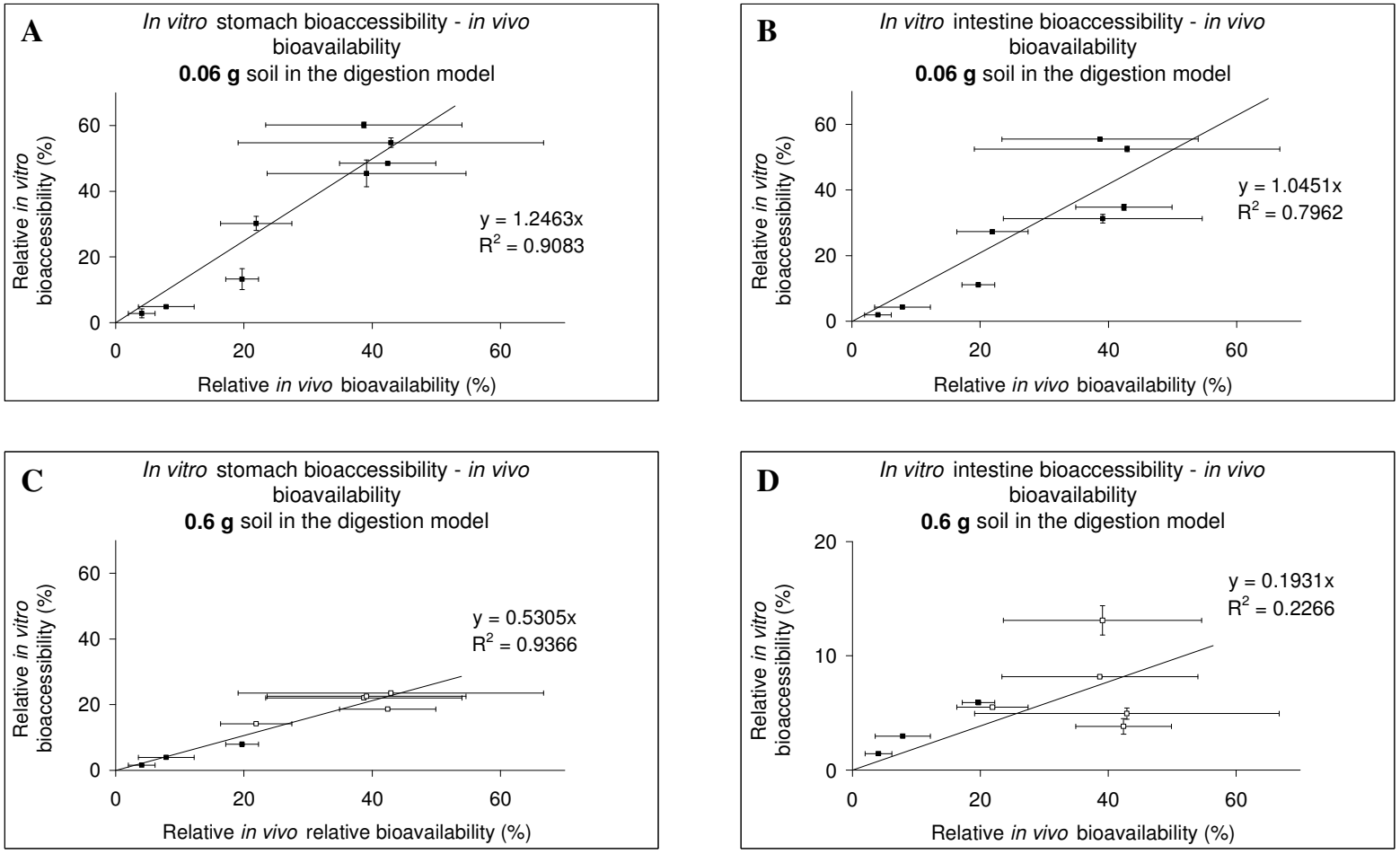

Figure 5. Correlation between the in vivo relative bioavailability and in vitro relative bioaccessibility for bioaccessibility determination in the stomach (A) and intestine (B) compartment with 0.06 g of soil in the digestion tube. Bioaccessibility was determined in the stomach (C) and intestine (D) with 0.6 g of soil in the digestion tube.

Open squares: gastric pH during in vitro digestion procedure was outside the allowed pH interval of 1 < pH < 2.

We expect that the slope of the oral bioavailability-bioaccessibility relationship is 1 for both the stomach and the intestinal compartment. For, the absorption of bioaccessible arsenic from soil and $Na_2AsO_4 \cdot 7H_2O$ should be equal. Also the metabolism of absorbed arsenic from soil and $Na_2AsO_4 \cdot 7H_2O$ should be equal. Hence:

$$\text{Slope}=\frac{\text{y-axis}}{\text{x-axis}}=\frac{Rel\ F_B}{Rel\ F}=\frac{\frac{F_{B,soil}}{F_{B,Na_2AsO_4\cdot 7H_2O}}}{\frac{F_{B,soil}}{F_{B,Na_2AsO_4\cdot 7H_2O}}\times\frac{F_{A,soil}}{F_{A,Na_2AsO_4\cdot 7H_2O}}\times\frac{F_{H,soil}}{F_{H,Na_2AsO_4\cdot 7H_2O}}}=\frac{\frac{F_{B,soil}}{F_{B,Na_2AsO_4\cdot 7H_2O}}}{\frac{F_{B,soil}}{F_{B,Na_2AsO_4\cdot 7H_2O}}\times 1\times 1}=1 \quad (20)$$

The slope is indeed approximately 1 in Figure 5A and B, which is the most appropriate figure to take. In Figure 5C and D only 3 valid data points are available, as the pH in most digestion tubes was outside the allowed range. As a consequence, the correlation in Figure 5C and D including all data is poor and the slope is not in line with expectations. On the contrary, the slope of 1 in Figure 5A and B correspond very well with the expected relationship between bioaccessibility and oral bioavailability. Hence, based on the good correlation between *in vitro* intestinal bioaccessibility and *in vivo* bioavailability (Figure 5B, r^2-value 0.796, 8 soils), and the agreement between theoretical and observed slope of the oral bioavailability-bioaccessibility relationship, ***we conclude that the bioaccessibility determined with the RIVM in vitro digestion model of arsenic-contaminated soils correlates well with the oral bioavailability determined in an juvenile swine study.*** Precondition is that the gastric pH ranges between 1 and 2, which was accomplished by using 0.06 g of soil per digestion tube. It is very well possible that a good correlation can be obtained for 0.6 g of soil per digestion tube, if the gastric pH is adjusted to the acceptable range. However, this has not been tested within the present study.

Although the correlation in Figure 5C is good, the outcome is still doubtful. First, the slope of the correlation is not 1, as is expected based on equation 20. Second, the gastric pH of most samples is above the acceptable range ($1 < pH < 2$). Finally, the correlation in the subsequent intestinal compartment is not good. Therefore, although the correlation gives a high r^2-value, this manner of bioaccessibility measurement (gastric bioaccessibility for digestion with 0.6 g soil per digestion tube) is not recommended to further use.
Besides the validation to the *in vivo* situation further experiments to quantify the reproducibility of the method are recommended.

6.2 Cadmium from soil

The Danish DHI, Christian Grøn and co-workers, implemented and validated among others the RIVM *in vitro* digestion model, and performed experiments with the model. One of the issues they investigated was the correlation between *in vitro* bioaccessibility determined by the RIVM fasted *in vitro* digestion model of cadmium-contaminated soil, and *in vivo* bioavailability determined in juvenile swine (Gron, 2005). This was determined for 14 soil samples. The correlation between *in vitro* bioaccessibility (y) and *in vivo* bioavailability (x) could be expressed as:

$$y = 0.93x + 0.039 \qquad (21)$$

The r^2-value of this correlation was 0.635, with an even distribution of data over the oral bioavailability range (Gron, 2005). Hence, also the bioaccessibility of cadmium determined by the RIVM fasted *in vitro* digestion model seems to give a satisfactory correlation with *in vivo* bioavailability data.

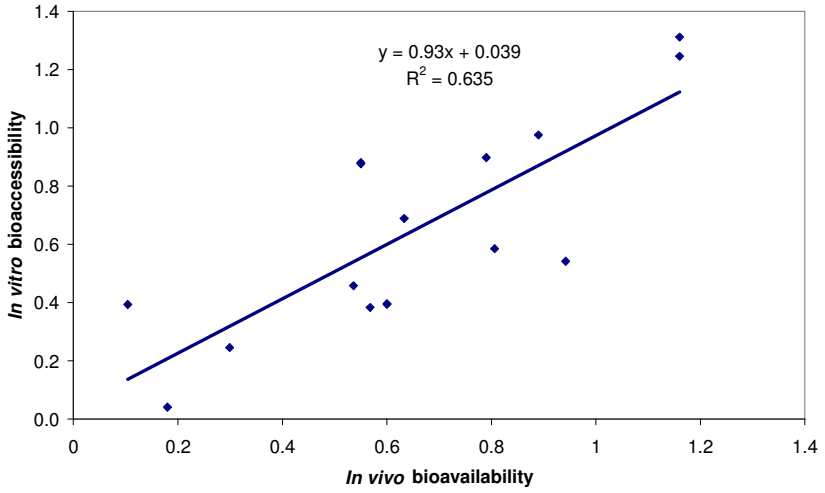

Figure 6. Correlation between in vivo bioavailability and in vitro intestinal bioaccessibility. Bioaccessibility is determined by DHI, Denmark according to the protocol of the RIVM in vitro digestion model with 0.6 g soil per digestion tube.
Figure published with permission of Christian Grøn (DHI, Denmark).

6.3 Ochratoxin A and aflatoxin B1 from food

In vitro bioaccessibility data of ochratoxin A and aflatoxin B1 from food were compared with measures of oral bioavailability of those compounds (Versantvoort *et al.*, 2005). Ochratoxin A and aflatoxin B1 are mycotoxins that are produced by fungi. The effect of modulating compounds, i.e. additions to the food that are known to reduce the toxicity to the mycotoxins, on the bioaccessibility is studied. The bioaccessibility with and without modulating compounds was compared to the information on the oral bioavailability and intestinal absorption obtained from literature. For example, the *in vivo* effect (a measure for the oral bioavailability) of ochratoxin A was reduced a factor 4-7 by addition of cholestyramine, whereas the bioaccessibility decreased from about 100% without to 12 ± 4% with addition of cholestyramine. The effect of the other modulating compounds is described in Table 5. These data indicate that the comparisons between *in vitro* bioaccessibility data and (measures of) oral bioavailability for ochratoxin A and aflatoxin B1 were satisfactory (Versantvoort *et al.*, 2005).
Detailed information can be found in Versantvoort *et al.* (2005).

6.4 Phthalates from PVC disks

Three models have been developed to assess the bioaccessibility of contaminants from toys and consumer matrices (Oomen *et al.*, 2003b; Oomen *et al.*, 2004c; Oomen *et al.*, 2005; Brandon *et al.*, in press). These latter models can simulate sucking and/or ingestion of the matrix. In one study the bioaccessibility of phthalates from PVC disks is studied. Phthalates are commonly used plasticizers for soft PCV to impart flexibility and durability. The release of a particular plasticizer, di-iso-nonyl phthalate or DINP, during sucking had been studied in humans and in the *in vitro* digestion model (Oomen *et al.*, 2004c). The DINP migration rate towards artificial saliva obtained with the RIVM *in vitro* digestion model (3.3 ± 0.5 µg/min) was in the same order of magnitude as the average of DINP release in saliva of human volunteers (1.4 µg/min). The RIVM method gave a slightly higher value than the average release rate for human volunteers, but is within the range of release rates found in the volunteer study (0.3-8.3 µg/min). Hence, also for the release of the phthalate DINP from PVC disks, the *in vitro* bioaccessibility data match with *in vivo* data.
Detailed information can be found in Oomen *et al.* (2004c).

6.5 Conclusions

Arsenic. The bioaccessibility of arsenic from 8 different soils obtained by the RIVM *in vitro* digestion model correlates well with *in vivo* relative bioavailability data obtained in an juvenile swine study. When 0.06 g soil per digestion tube was used, gastric pH values were within the allowed range (1 < pH < 2). Because there is a scientific basis for the relationship between intestinal bioaccessibility and bioavailability, bioaccessibility in the intestine is preferred over gastric bioaccessibility, in which case an r^2-value of 0.796 is obtained (Figure 5B). Hence, conditions to test arsenic bioaccessibility from soil are: 0.06 g of soil per digestion tube, 1 < gastric pH < 2, and determination of intestinal bioaccessibility. Further research should verify whether using 0.6 g of soil per digestion tube and when necessary adjustment of the gastric pH also results in a satisfactory correlation. Larger amounts of soil per digestion tube than 0.06 g are desirable so that a more representative sample can be taken. Yet, in the present research duplicates of runs with 0.06 g soil per digestion tube were reproducible.

Cadmium, ochratoxin A, aflatoxin B1, and phthalate. Also the bioaccessibility of cadmium from soil (investigated by Christian Grøn and co-workers, DHI Denmark), ochratoxin A and aflatoxin B from food with and without modulating compounds, and the release of phthalate from PVC disks towards saliva obtained with the RIVM *in vitro* digestion model correspond well with (measures) of *in vivo* bioavailability.

*Table 5. Results of **aflatoxin B1** and **ochratoxin A** obtained with RIVM fed in vitro digestion model and Caco-2 intestinal transport compared to in vivo data in humans and animals. Detailed information can be found in Versantvoort et al. (2005).*

Compound	**Species**	***In vivo* effect**	***in vitro* digestion model**	**Intestinal transport Caco-2 cells**	***in vitro-in vivo* correlation**		
					Digestion model	**Caco-2 cells**	**Overall correlation**
Aflatoxin B1	Rat	High absorption	112 ± 14 %#	$8.6x10^{-6}$ cm/s	+	+	+
+ chlorophyllin	Human	2x↓	107 ± 12 %	$0.4x10^{-6}$ cm/s	-	+	+
+ activated charcoal	Poultry, goat	>4x↓*	1 ± 2 %	n.m.	+		+
+ HSCAS (Myco-AD®)	Rat, poultry, pig	~4x↓	15 ± 13 %	n.m.	+		+
+ cholestyramine	No information	no information	71 ± 8 %	n.m.	?		?
Ochratoxin A	Rat, mouse	High absorption	111 ± 20 %#	n.d.	+		+
+ chlorophyllin	No information	No information	93 ± 23 %	n.d.	?		?
+ activated charcoal	Pig, rat	~5x ↓	12 ± 4 %	n.m.	+		+
+ HSCAS (Myco-AD®)	Pig, poultry	↔	103 ± 13 %	n.m.	+		+
+ cholestyramine	Rat	4-7x ↓	12 ± 4 %	n.m.	+		+

n.m. not measured.

n.d. could not be determined. Transport of lead across Caco-2 cells could not be determined reliably because the transport filter itself formed a barrier. Transport of ochratoxin A could not be determined because ochratoxin A could not be recovered from the transport medium probably due to a very tight binding of ochratoxin A to bovine serum albumin.

* Mortal effects of aflatoxin were completely abolished in presence of high concentrations of activated charcoal.

Bioaccessibility of aflatoxin B1 and ochratoxin A was determined from 2 different food-mixes consisting of 4.5 g infant formula, 0.5 g peanut slurry containing 6 and 12 ppb, and 1 g buckwheat containing 11.4 ppb.

7. Conditions in the *in vitro* digestion model that should be used for application in risk assessment

The RIVM *in vitro* digestion model allows adaptation to exposure scenarios, which can be useful in risk assessment. Presently, different variables that can affect the bioaccessibility results are addressed:

1. the physiological state that is simulated (fed versus fasted),
2. evaluation of both gastric and intestinal bioaccessibility, and
3. the soil-to-solution ratio.

This leads to a set of recommendations for the conditions to can be used for implementing bioaccessibility in risk assessment.

7.1 Physiological state

The bioaccessibility of lead from several soils is determined for two physiological states, that is, **fasted** and **fed conditions**. Fasted conditions are simulated by the *in vitro* digestion model developed in the present project (Oomen *et al.*, 2003a), see section 3.2.1. Fed conditions are simulated by the *in vitro* digestion model developed in project " *in vitro* digestion model food/toy" (Versantvoort *et al.*, 2004), see section 3.2.2 (Versantvoort *et al.*, 2005). Due to the presence of enzymes in the digestion juices the food is (partly) degraded, similar to the *in vivo* situation. We aim to investigate the degradation of food by these enzymes in the system in the future. By comparing the bioaccessibility data of the same soils for both fasted and fed conditions, insight is obtained in the magnitude of the difference in bioaccessibility due to the physiological state. This enables a decision on which physiological state should be simulated by the *in vitro* digestion model.

As shown in Figure 7, bioaccessibility of lead for fasted conditions is higher than bioaccessibility of lead for fed conditions, i.e. fed conditions simulated in the digestion model and in the presence of breakfast or spaghetti meal. Based on the experiments shown in Figure 7, e.g. 12 soils, the difference between fed and fasted bioaccessibility amounts on average a factor 0.56 ± 0.20. Fed conditions were obtained by addition of a meal, e.g. a breakfast or a spaghetti meal, and the *in vitro* digestion model simulating fed conditions. Also from literature, it is known that lead is better absorbed in fasted conditions and, hence, better bioavailable, than in fed conditions (James *et al.*, 1985; Heard *et al.*, 1983; Heard *et al.*, 1982; Blake *et al.*, 1983). Based on the higher bioaccessibility and higher absorption of lead in fasted conditions, **the fasted state is the most conservative state to assess a relative bioavailability factor of lead from soil**, i.e. results in a higher relative bioavailability factor than for fed conditions. Hence, **the choice of the physiological condition that is used for**

estimation of the bioavailable lead fraction highly influences the outcome. In order to estimate an oral bioavailability value for lead after soil ingestion for an average physiological condition, **a weighted value for the bioavailability based on both fasted and fed conditions is recommended**. On the other hand, estimating a weighted bioavailability based on both fasted and fed conditions requires knowledge on the bioaccessibility for both fasted and fed conditions. Due to limited information on the bioaccessibility of lead from soil for fed conditions (12 soils), we cannot derive a reliable default factor for the relationship between bioaccessibility of lead for fasted and fed conditions. Hence, at present, not enough information on the relationship between fasted and fed bioaccessibility is available to estimate the bioaccessibility for the average physiological state based on the fasted bioaccessibility only.

An option to come to a weighted bioaccessibility value is that the bioaccessibility of lead from soil is determined by two different *in vitro* digestion models, e.g. for fasted and fed conditions. This requires twice as high costs and time as determination of the bioaccessibility for fasted conditions. Therefore, it can also be chosen to determine the bioaccessibility only for fasted conditions, resulting in a conservative value, but with less experimental costs. Finally, further research on the relationship between bioaccessibility for fasted and fed conditions can be performed. Note, the choice of the physiological state to determine the bioaccessibility or the weighting of the physiological states for implementation of bioaccessibility into risk assessment is also a matter of policy.

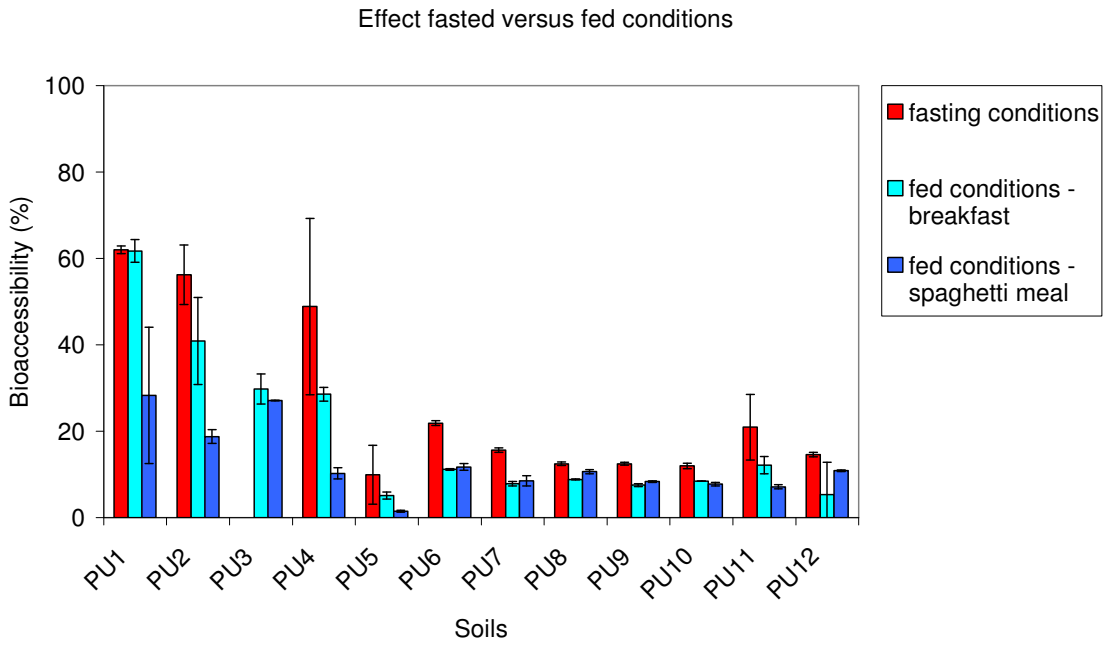

Figure 7. The bioaccessibility of lead from historically contaminated soils (PU1-PU12) in the intestinal compartment for fasted conditions, and fed conditions in the presence of a high fat breakfast or a spaghetti meal. The standard deviations are indicated by the vertical lines.

In order to calculate a weighted value for the oral bioavailability of lead from soil based on both fasted and fed conditions, an estimate should be made of the time a child is fasted and fed. If a child eats 3 meals per day, and conditions can be considered fed for 2 hours after the meal, the child is fed for 6 hours per day. It can be assumed that a young child (1-4 years) sleeps 12 h per day, which is not included as potential play time, but merely indicates that the potential play time during a day is the other 12 h. Hence, **the child is assumed to be in a fed state for 6 of the 12 h, or half of the time, and in the fasted state for the other half of the time**. Note that it likely that the child is fed for more than half of the time as a child usually has a snack in between the meals. This indicates that the present estimation for fed and fasted time is likely to be conservative, but acceptable due to the lack of information on this issue.

7.2 Gastric versus intestinal bioaccessibility

The bioaccessibility of lead from soil has been determined both in the stomach and intestine. As can be seen in Figure 8, the bioaccessibility for gastric conditions is higher than for intestinal conditions. Bioaccessibility in the stomach was also higher for fed conditions, in the presence of a high-fat breakfast or a spaghetti meal (data not shown). Based on the data shown in Figure 8, the gastric bioaccessibility is about twice as high as the intestinal bioaccessibility (2.2 ± 0.7). Since absorption of lead takes place in the intestine (IPCS, 1995), the bioaccessibility determined in the intestinal compartment is expected to give the most realistic value for estimation of the oral bioavailability of lead. **Hence, when bioaccessibility determined in the stomach compartment instead of the intestinal compartment is used, the actual risk for humans is probably overestimated, based on data of the RIVM *in vitro* digestion model by about a factor 2. In addition, there is a scientific basis for the relationship between bioaccessibility in the intestine and bioavailability. Therefore, we recommend using intestinal bioaccessibility for application in risk assessment.**

Several groups use *in vitro* digestion models that only simulate the conditions in the stomach, and thus only obtain gastric bioaccessibility values. As this is not the physiological compartment where absorption of lead, and most other compounds, takes place, these models can only operate on the basis of correlation between *in vitro* bioaccessibility data and *in vivo* bioavailability data. This indicates that for each new situation, i.e. other compounds, other soil types, other lead speciation, the correlation should be established before the method can be applied in risk assessment. Although a good correlation between *in vitro* and *in vivo* data is also essential for the RIVM *in vitro* digestion model, there is a physiological basis for the bioaccessibility obtained by the RIVM *in vitro* digestion model and the oral bioavailability, i.e. $F_B \times F_A \times F_H$ (see section 2.2). The scientific basis of the RIVM model was verified by the comparison of *in vitro* bioaccessibility with *in vivo* bioavailability data, chapter 5 and 6, both by the linear correlation, but also by the slope of the correlation that was in agreement with theoretical expectations. Therefore, it is more likely that the RIVM model gives a just result

for conditions that are not fully covered by the validation to the *in vivo* situation compared to stomach models, i.e. different soil type, lead speciation, or perhaps even different compounds. The general applicability of the RIVM *in vitro* digestion model is also stressed by the good accordance between *in vitro* and *in vivo* data for other compounds, i.e. arsenic, cadmium, ochratoxin A, aflatoxin B1, and phthalate, as described in chapter 6.

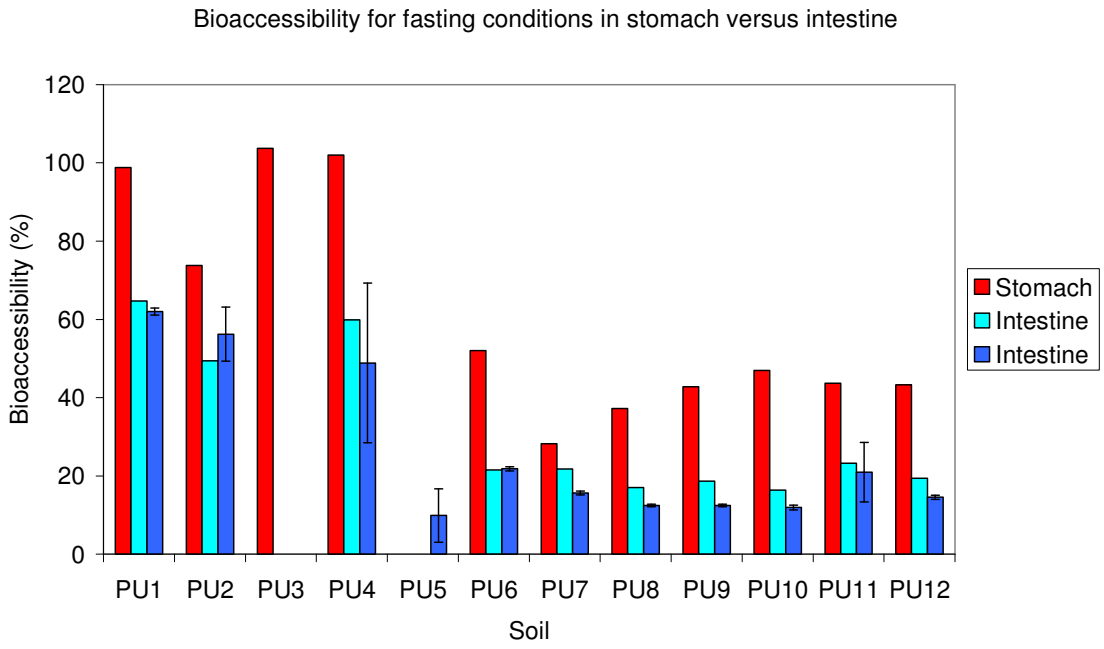

Figure 8. Bioaccessibility of lead from historically contaminated soils (PU1-PU12) in the stomach (n=1) and intestinal compartment. The two different data sets for the intestine represent two different experimental series with n=1 and n=2, respectively. The standard deviations are indicated by the vertical lines.

When bioaccessibility determined for gastric conditions would be used in risk assessment without the use of the relationship obtained in the *vitro-vivo* correlation, the risks of lead from soil are probably overestimated. Based on gastric and intestinal bioaccessibility data obtained with the RIVM *in vitro* digestion model, the bioaccessibility would be overestimated by about a factor 2. Obviously, this factor might be different for other *in vitro* digestion models and contaminants, for example due to another gastric pH value.

7.3 Soil-solution ratio

For calculation of the soil-solution ratio that is to be used in the *in vitro* digestion model, we assume that children ingest **100 mg soil per day** via hand-to-mouth behaviour

(Lijzen *et al.*, 1999). According to decisions made in Dutch politics, this amount of daily ingested soil is deduced for “an average child”. This means that the deduction of the amount of daily ingested soil is based on children showing normal hand-to-mouth behaviour, and not on so-called pica behaviour in which case the child deliberately ingests soil.

The gastric volume in fasted conditions is 50 ml for adults and 9 ml for children (Kulkarni *et al.*, 1997; Geigy, 1969; Davenport, 1984; Kawana *et al.*, 2000). In the calculation of the soil-solution ratio we continue our calculation for children only, as they are more vulnerable for adverse effects due to lead exposure than adults, going for the scenarios leading to the highest and lowest soil-solution ratios.
The lowest soil-solution ratio is when assuming that 100 mg of soil is ingested at once, in which case the soil-solution ratio in the stomach would be 1:90. However, children ingest the soil throughout the day. When 100 mg of soil is ingested at even rate over 12 h (assuming that the child sleeps the other 12 h), 8 mg of soil is present in 9 ml of gastric juice, which results in a solid-to-fluid ratio of 1:1125. Since the gastric juice is also renewed (about every 20 min), the soil-solution ratio can be even higher, i.e. 1:>1125. Hence, the actual solid-to-fluid ratio for hand-to-mouth behaviour lies somewhere between 1:90 and 1:>1125.
In the present model we have used both 0.6 g and 0.06 g per digestion tube. This results in a soil-solution ratio of 1:38 and 1:375 in the stomach, and 1:98 and 1:975 in the intestine, respectively. Hence, 0.6 g per digestion tube results in a soil-solution ratio in the stomach (1:38) that is above the range derived above for hand-to-mouth behaviour (1:90 up till 1:>1125). Yet, 0.6 g per digestion tube has as advantages that 1) the lead in digestive juice can be easily analysed, and 2) a representative soil sample can be taken. With less soil per digestion tube the chances are higher that the outcome becomes less reproducible due to inhomogeneity of the soil samples, although up till now we have found reproducible bioaccessibility values for 0.06 g soil per digestion tube. Furthermore, with 0.06 g per digestion tube, the pH in the stomach and intestinal compartment is only slightly affected by the presence of soil. For some soils, for digestions with 0.6 g per digestion tube, the pH rose as high as 4 in the stomach, leading to bioaccessibility values that are not representative and that do not correlate with the *in vivo* bioavailability values (see section 5.3). In addition, the soil-solution ratio of 0.06 g soil per digestion tube (1:375) is in the middle of the possible soil-solution ratio range of 1:90 and 1>1125. Therefore, **we recommend to use 0.06 g soil per digestion tube.**
If possible, further research on the possibilities of 0.6 g (1:38) or 0.2 g (1:114) per digestion tube are recommended, with pH correction in the stomach in case the pH rises too much (>2). It should be checked that if the pH in the stomach is adjusted to within the allowed pH-interval, the bioaccessibility data still correlate to the *in vivo* bioavailability data.

7.4 Conclusions

Taken the variables addressed above together, we recommend the following conditions to be used in the *in vitro* digestion model when bioaccessibility is to be implemented into risk assessment:

- A **weighted value** of the bioaccessibility of lead from soil based on **both fasted and fed conditions** is recommended for simulation of a realistic value for oral bioavailability. A default relationship between bioaccessibility determined for fasted and fed condition can at the moment not be derived due to a limited data set. If information on the bioaccessibility for the average physiological state is necessary, the bioaccessibility has to be determined twice: in an *in vitro* digestion model simulating fasted and fed conditions. Alternatively, the most conservative bioaccessibility value can be determined (fasted conditions) and used to estimate the oral bioavailability of lead from soil. Another option is further research to estimate the weighted bioaccessibility value based on one experiment, i.e. investigate the relationship of the bioaccessibility of lead from soil between fasted and fed conditions. **Policy makers should give directions** in the choice whether a realistic situation, but (initially) more expensive solution is preferred in risk assessment over a conservative, but cheaper approach.
- Bioaccessibility should be determined in the **intestinal compartment** as absorption of lead takes place in the intestine. Bioaccessibility determined in the stomach compartment can result in a physiologically not relevant relative bioavailability factor, that overestimates the actual bioaccessibility. When bioaccessibility is not determined in line with physiology, the method can only be applied after empirical correlation with *in vivo* data. Since the stomach model is less similar to human physiology, the likelihood that a wrong bioaccessibility value is determined is greater than for more physiologically based models that also include an intestinal compartment.
- For the time being, an amount of 0.06 g per digestion tube is recommended for the *in vitro* digestion of a certain soil. Research is recommended to check whether 0.6 or 0.2 g soil per digestion tube, and pH adjustment when necessary, can also be used.

8. Soil

Obviously, both the soil and the physicochemical conditions of the *in vitro* digestion model affect the bioaccessibility. In the present chapter we address 1) the effect of the lead concentration in soil on bioaccessibility, and 2) the relationship between soil characteristics and bioaccessibility of lead from soil.

8.1 Effect contamination level soil on bioaccessibility

Soils can be contaminated at any level. Also within a contaminated site, one is often confronted with a range of different contamination levels. If the bioaccessibility of lead is dependent on the contamination level, this would lead to some practical difficulties: which bioaccessibility should be used to calculate the relative bioavailability factor? Therefore, we studied the relationship between the contamination level and the bioaccessibility of lead.

The concentration range at which (urgent) remediation might be necessary is the concentration range for which implementation of oral bioavailability into risk assessment is relevant. This is considered to be a contamination level of up to 5 times the Intervention Value. For sites with contamination levels higher than 5 times the Intervention Value the risks are considered to be that high that there is urgency for remediation, regardless the bioaccessibility. The present Intervention Value of lead in soil in the Netherlands is 530 mg/kg (Swartjes, 1999). Hence, we studied the relationship between the contamination of four Dutch soils spiked with lead (0-2650 mg/kg dry weight) against the bioaccessibility. The gastric and intestinal bioaccessibility of lead were independent of the contamination level of the soils, i.e. the same bioaccessibility (%) was obtained for all contamination levels, see Figure 9. Hence, it is concluded that the **lead concentration in soil does not significantly affect the bioaccessibility in the concentration range 0-2650 mg lead/kg dry soil**. This indicates that within a site with similar soil characteristics, the bioaccessibility of lead (expressed as %) can be assumed to be the same, regardless the contamination level of the soil (0-2650 mg lead/kg dry soil).

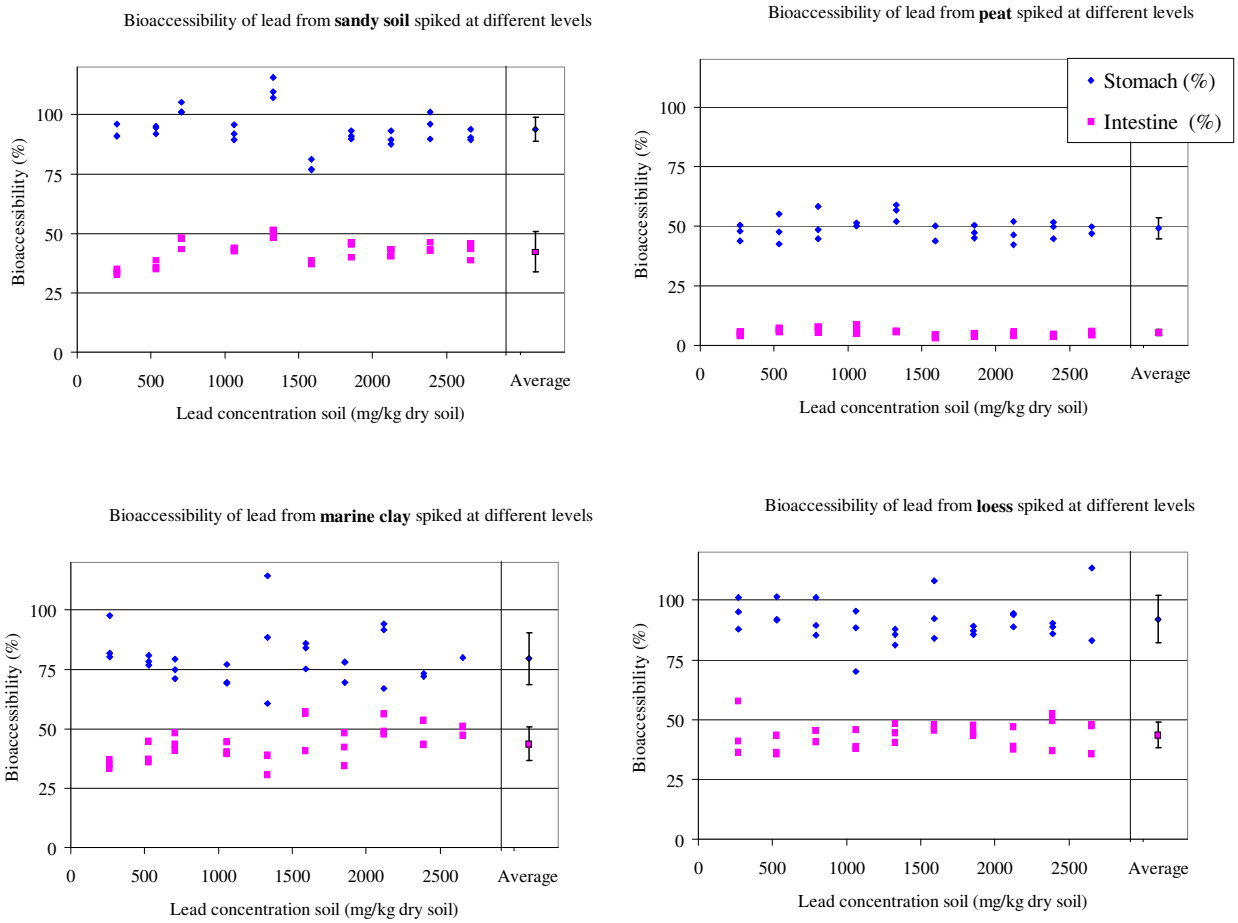

Figure 9. Relationship between the contamination level of lead in 4 spiked Dutch soils and the bioaccessibility (expressed as %). Gastric bioaccessibility data are presented as blue diamonds, whereas intestinal bioaccessibility are presented as pink squares. The average bioaccessibility and standard deviation are shown at the right of each sub-figure for each soil.

8.2 Relationship between bioaccessibility and soil characteristics

The aim of the present research was to investigate **whether a relationship between bioaccessibility and soil characteristics exist**. Ultimately, such a relationship can be used to make a rough, conservative estimate of the bioaccessibility of lead from soil based on a few soil characteristics. Hence, investigation of the relationship between bioaccessibility and soil characteristics can be seen as the first steps towards *in silico* modelling of bioaccessibility. This can be used in an early phase of risk assessment of a specific site, and help in the decision for further (experimental) research on oral bioavailability.

8.2.1 Scientific background

Soil characteristics and bioaccessibility of lead were determined from historically contaminated soils (n=43). These soils consist of several series: about half of the soils were obtained in the Netherlands, mostly in the Province of Utrecht, which were studied partly by the RIVM alone, and partly in a co-operation with Utrecht University (Nikolaj Walraven). 25 Soils (sieved to the fraction <50 µm) were studied in a co-operation with the Swedish University of Agricultural Sciences (Karin Ljung), and 5 soils that were used in the *vitro-vivo* correlation for arsenic (section 6.1) were also analysed successfully for lead. In addition, some other isolated Dutch soils were included. These soils were all processed in the RIVM *in vitro* digestion model at RIVM.

Figure 10 shows a trend between the bioaccessibility and the percentage organic matter of these soils. Soils that are low in organic matter such as sandy soils can have high bioaccessibility values, although low bioaccessibility is possible too. On the other hand, soils high in organic matter show in general lower bioaccessibility values. The low bioaccessibility values found with low organic matter content of the soil can be explained in many different ways. Besides the organic matter, other soil characteristics probably also affect the bioaccessibility, and the speciation of lead is probably also of importance (Ruby *et al.*, 1999). In some cases lead has been associated to pottery chips, resulting in very low bioaccessibility (Oomen *et al.*, 2003a), and minimizing the effect of soil characteristics on bioaccessibility. Bioaccessibility of lead from shooting range appeared to be associated with high bioaccessibility (organic matter 2.1 and 3.0%, bioaccessibility 65 and 49%, respectively), but this may be different if actual bullets were taken for the bioaccessibility experiment.

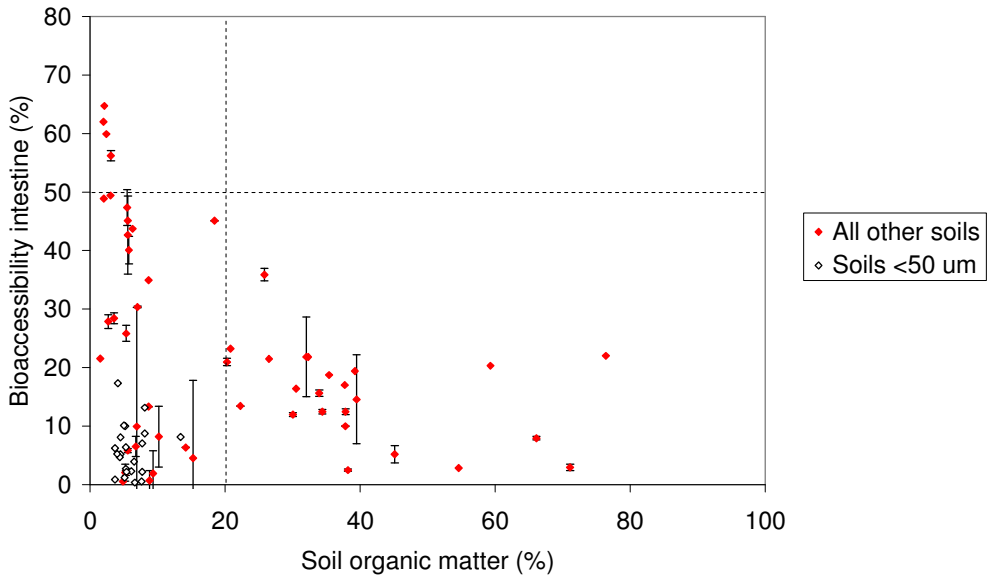

Figure 10. Intestinal bioaccessibility (fasted conditions) of lead from historically contaminated soils versus the organic matter content of the soils.

The data set is presently not large enough to qualitatively identify with multiple regression statistics all soil and lead speciation characteristics that are determining the bioaccessibility. Yet, it is clear that soil organic matter is an important characteristic.

8.2.2 Application into risk assessment

A manner to make a simple estimation of the (maximum) bioaccessibility of lead from soil based on soil characteristics would be very useful in risk assessment. Such information can be applied as a first step towards site-specific risk assessment without the requirement of additional experimental data, see also chapter 12. Therefore, it is recommended to extent the present data base, and perform multiple regression on the extended data base.
However, in order to make a first step towards such a relationship, a pragmatic approach is proposed for the time being. **We recommend using a default bioaccessibility value for all soil with less than 20% organic matter, and a default bioaccessibility value for all soil with more than 20% organic matter.** This default bioaccessibility value can be, depending on the choice of Dutch policy makers, the bioaccessibility value that represent the 80^{th}, 85^{th}, 90^{th}, or 95^{th} percentile of all relevant bioaccessibilities in the applicable organic matter window. Table 6 presents the bioaccessibility values associated with the different percentiles. The default bioaccessibility values correspond to a default relative oral bioavailability factor. The default relative bioavailability factor for lead is calculated with the relationship between *in vitro* bioaccessibility (RIVM model) and bioavailability as described in chapter 9.

As can be seen in Table 6, the default relative oral bioavailability factor is >1 for soils with an organic matter content <20% at the 95^{th} percentile. This indicates that, according to the present calculation with the present assumptions, the health risks after ingestion of some soils is greater than based on dietary lead.
Furthermore, it can be seen in Table 6 that the 80^{th}-95^{th} percentile of the default relative bioavailability factor for soils low in organic matter is close to 1 (0.87-1.20). This indicates that for these soils, the default relative bioavailability factor cannot or only slightly influence the value of the calculated daily exposure of lead from soil. As most Dutch soils are low in organic matter, we recommend to keep the relative bioavailability factor of 1 in the derivation of the Intervention Value of lead in soil. In contrast, for soils known to be high in organic matter, for example “toemaakdekken” a lower relative bioavailability factor might be applied.

Table 6. Default bioaccessibility values and relative bioavailability factors associated with different percentiles of the bioaccessibility of lead from historically contaminated soils (bioaccessibility determined for fasted conditions).

Percentile	**Organic matter < 20%**		**Organic matter > 20%**	
	Default **bioaccessibility** value	Default **relative bioavailability factor**	Default **bioaccessibility** value	Default **relative bioavailability factor**
P50	20.4	0.41	10.9	0.22
P80	43.7	0.87	20.8	0.42
P85	43.9	0.88	21.1	0.42
P90	48.6	0.97	21.4	0.43
P95	59.9	1.20	23.3	0.47

The data of soils that were sieved to <50 µm were excluded, as this is not representative for the standard situation. Also bioaccessibility values <1% were excluded as these bioaccessibility values can probably be explained by specific causes, i.e. lead in pottery flakes etc. Averages were used for soils that were tested in duplo, triplo, or on several days, so that each soil was equally important in the derivation of the default bioaccessibility value.
The data were not log-normalized as it is not expected that a specific fraction of all soils would lead to a normal distribution of bioaccessibility values. The derivation of the default bioaccessibility values for organic matter < 20% is based on 25 data points, for organic matter > 20% based on 18 data points.

8.3 Conclusions

The lead concentration in soils of constant characteristics does not significantly affect the bioaccessibility in the concentration range 0-2650 mg lead/kg dry soil. Hence, for a particular site with similar soil characteristics, the bioaccessibility (expressed as %) can be assumed to be the same within the concentration range of 0-2650 mg lead/kg dry soil.
A trend is observed between bioaccessibility and percentage soil organic matter, with lower bioaccessibility values at high organic matter. The data set of historically contaminated soils is not large enough to identify with multiple regression the soil characteristics that are determining bioaccessibility. Therefore, for the time being, a pragmatic approach is proposed with a default bioaccessibility value for soils of organic matter content <20% and >20%.The default bioaccessibility value can be converted to a default relative oral bioavailability factor. The values for the various default relative oral bioavailability factors are respectively 0.87, 0.88, 0.97, and 1.20 for the 80^{th}, 85^{th}, 90^{th}, and 95^{th} percentile of soils <20% organic matter. For soils with an organic matter content >20%, the default relative bioavailability values for the 80^{th}, 85^{th}, 90^{th}, 95^{th} percentile are respectively 0.42, 0.42, 0.43, and 0.47. This implies that for default relative bioavailability factors <1, the calculated exposure to lead via soil ingestion is less than according to the present calculation in CSOIL. However, for soils low in organic matter (<20%) the default relative bioavailability factor is close to 1, indicating

that we recommend to keep the relative bioavailability factor of 1 in the derivation of the Intervention Value of lead in soil.

9. Relative bioavailability factor in exposure modelling

The *in vitro* determined bioaccessibility of lead from soil should be related to a relative bioavailability factor, i.e. the factor accounting for the difference in oral bioavailability of dietary lead and lead from soil. This relative oral bioavailability factor (*Rel* F) can be implemented into risk assessment for exposure of lead by a human being via ingestion of soil (and house dust) according to the CSOIL methodology (Otte *et al.*, 2001):

$$DI = \frac{AID \times C_s \times Rel\ F}{W} \qquad (22)$$

With:
- DI: uptake via ingestion ($mg_{contaminant} \times kg^{-1} \times d^{-1}$)
- AID: daily intake soil/house dust via ingestion ($kg \times d^{-1}$)
- W: body weight (kg)
- *Rel* F: relative oral bioavailability factor, presently set at 1 (-)
- C_s: Concentration contaminant in soil/house dust ($mg_{contaminant} \times kg^{-1}$)

In the present section this relationship between *in vitro* bioaccessibility determined with the RIVM model and the relative bioavailability factor is addressed.

The relative bioavailability factor (*Rel* F) for lead from soil is the bioavailability of lead from soil (F_{soil}) divided by the bioavailability of lead from the matrix that was used for deduction of the Maximum Permissible Risk (MPR_{human}), i.e. F_{MPR}, see also chapter 2. The relative F is the ratio of the two bioavailability values:

$$Rel\ F = \frac{F_{soil}}{F_{MPR}} \qquad (23)$$

Three processes within oral bioavailability are distinguished, see Chapter 2: bioaccessibility (F_B), intestinal absorption (F_A), and metabolism (F_H). Equation 23 can thus be reformulated to:

$$Rel\ F = \frac{F_{B,\,soil} \times F_{A,\,soil} \times F_{H,\,soil}}{F_{B,MPR} \times F_{A,MPR} \times F_{H,MPR}} \qquad (24)$$

In contrast to the study of Bunker Hill soil in adults by comparison between *in vitro* bioaccessibility data and *in vivo* bioavailability data of lead in Bunker Hill soil (section 5.2), the derivation of the relative bioavailability should focus on **children**. Children are

considered to be the group at risk for lead intoxication. This choice has important implications for the calculation of the relative bioavailability factor.

Below, information on the bioavailability of lead from the matrix used in the MPR studies is first addressed, e.g. the denominator in equation 23 and 24. Subsequently, the bioavailability of lead from soil is investigated, e.g. the numerator in equation 23 and 24. Put together this leads to a relationship between bioaccessibility and bioavailability of lead from soil.

9.1 Bioavailability of lead from matrix used in MPR_{human} studies

Dutch risk assessment for lead is based on a criterion laid down by the FAO/WHO (1993) and the IPCS (1995). The recommendation is to avoid lead blood levels above 50 µg/l, resulting in a provisional tolerable weekly intake (PTWI) of 25 µg/kg body weight per week, i.e. or a Tolerable Daily Intake (TDI) of 3.6 µg/kg body weight per day (Baars *et al.*, 2001). This criterion is based on ingestion of 3-4 µg lead/kg body weight/day by children, which was not associated with an increase in blood lead concentration. In addition it was indicated that the lead absorption in this study was on average 40%, i.e. $F_{B,MPR} \times F_{A,MPR} = 0.4$ (IPCS 1995; FAO/WHO 1993; Baars *et al.*, 2001; Ziegler *et al.*, 1978; Ryu *et al.*, 1983). As lead is not metabolised in the human body, i.e. $F_H=F_{H,soil}=F_{H,MPR}=1$, the relative F can be calculated by:

$$Rel\ \mathrm{F} = \frac{\mathrm{F}_{soil}}{\mathrm{F}_{MPR}} = \frac{\mathrm{F}_{B,soil} \times \mathrm{F}_{A,soil}}{0.4} \qquad (25)$$

9.2 Bioavailability of lead from soil

Values for bioaccessibility ($F_{B,soil}$) and absorption ($F_{A,soil}$) of lead from soil should be obtained for **conditions representative for episodes in which soil is ingested by children**. Children will ingest soil both in fasted and fed condition.
In case of **fasted conditions**, oral bioavailability of lead from soil will be higher than for fed conditions since 1) the **bioaccessibility** for fasted conditions is higher than for fed conditions (see section 7.1), and 2) the **absorption** of bioaccessibility lead for fasted conditions is higher than for fed conditions. The latter is possibly due to the competition of lead with calcium and other food component for absorption, see section 2.1. Hence, assuming fasted conditions for the calculation of the relative bioavailability (*Rel* F) would not be representative for the average exposure of a child to lead from soil ingestion. **Therefore, we recommend using a**

weighted value for the bioavailability of lead from soil based on both fasted and fed conditions.

In order to calculate a weighted value for the oral bioavailability of lead from soil based on both fasted and fed conditions, an estimate should be made of the time a child is fasted and fed. A child is assumed to be fed for 6 of the 12 h, or half of the time, and fasted for the other half of the time, see section 7.1.

9.2.1 Bioaccessibility of lead from soil

In order to obtain a value for the oral bioavailability of lead from soil based on both fasted and fed conditions, a weighted value for the bioaccessibility of lead from soil should be derived. It is clear that the bioaccessibility of lead from soil for fed conditions is lower than for fasted conditions, see section 7.1. For the 11 soils tested, each with 2 different foods added in the digestion, the factor between bioaccessibility for fed and fasted conditions was 0.56 ± 0.20:

$$F_{B,soil,fed} = 0.56 \times F_{B,soil,fasted} \quad (26)$$

However, as this relationship was based on 11 soils only, the factor 0.56 is not reliable enough to be used generally. Hence, estimation of the bioaccessibility for the average physiological condition based on one experimentally determined bioaccessibility value is presently not possible. The bioaccessibility of a particular soil can be measured both for fasted and fed conditions, after which the weighted bioaccessibility of lead from soil can be calculated by:

$$F_{B,soil} = \frac{F_{B,soil,fasted} + F_{B,soil,fed}}{2} \quad (27)$$

However, this requires a doubling of the *in vitro* experiments, and thus an increase in time and costs, as bioaccessibility has to be determined for both fasted and fed conditions. Determination of the bioaccessibility for fasted and fed conditions has not yet been done for many soils. Therefore, we have used the **bioaccessibility of lead for fasted conditions in further calculations**. The bioaccessibility of lead for fasted conditions is thus a conservative value. However, for future assessment of the relative oral bioavailability of lead from historically contaminated soils the determination of the bioaccessibility of lead for both fasted and fed conditions is possible, and will lead to a more realistic estimate of the bioaccessibility for the average physiological situation of a child. In addition, with further research on the bioaccessibility of lead from historically contaminated soils for both fasted and fed conditions, it might be possible to derive a relationship between fasted and fed conditions which can be used to derive a more realistic default relative bioavailability factor. If the present factor between bioaccessibility of lead from soil for fasted and fed conditions is true

(presently only based on limited data-set of 11 soils), the bioaccessibility for average physiological conditions is about 80% of the bioaccessibility for fasted conditions.

9.2.2 Estimation of the absorption of bioaccessible lead for children

Children are known to absorb lead to a larger extent than adults do (O'Flaherty, 1995; Mushak, 1991). This is probably due to the higher calcium demands and calcium uptake necessary for rapid bone formation. Hence, for children, other F_A values apply than for adults. The absorption of bioaccessible lead for fed conditions ($F_{A,adult,fed}$) is 4-18% for adults, see section 5.2.2. In the present calculation the absorption of dietary lead ($F_B \times F_A$) for small children is assumed to be 40%, as this figure is also assumed in the studies upon which the MPR is based, see section 9.1 (IPCS 1995; FAO/WHO 1993; Baars *et al.*, 2001). The bioaccessibility of lead from food determined by the RIVM *in vitro* digestion model ranged between 50 and 80%, and was on average 65%. Hence, a bioaccessibility of 65% of lead from food for fed conditions is used for calculation of the absorption of bioaccessible lead for fed conditions ($F_{A,fed,children}$):

$$F_{fed,children} = F_{B,fed,children} \times F_{A,fed,children} \quad (28)$$

$$0.4 = 0.65 \times F_{A,fed,children} \quad (29)$$

$$F_{A,fed,children} = \frac{0.4}{0.65} = 0.615 \quad (30)$$

Hence, **61.5% of bioaccessible lead from food is considered to be absorbed by children**. Unfortunately, no lead absorption data for small children for fasted conditions are available. As absorption of lead for adults is higher for fasted conditions (30-70%) than for fed conditions (4-18%), absorption of bioaccessible lead by small children for fasted conditions can also be considered to be higher than 61.5%. Therefore, in the present calculation, **100% absorption of lead by children for fasted conditions ($F_{A,fasted,children}$=1.0) is used as a worst case assumption**.

The weighted absorption factor of bioaccessibility lead $F_{A,average,children}$ for children, i.e. based on half of the time fed and half of the time fasted conditions, is thus:

$$F_{A,average,children} = \frac{F_{A,fasted,children} + F_{A,fed,children}}{2} = \frac{1.0 + 0.615}{2} = 0.8 \quad (31)$$

This indicates that the absorption of bioaccessible lead for the average situation in children is 80%.

9.2.3 Relative bioavailability factor

With knowledge about the bioavailability of lead in the MPR studies (section 9.1) and about the absorption of bioaccessible lead for the average situation by children (subsection 9.2.2), the relationship between relative bioavailability (*Rel* F) and bioaccessibility of lead from soil becomes:

$$Rel\ \mathrm{F} = \frac{\mathrm{F_{B,soil}} \times \mathrm{F_{A,average,children}}}{\mathrm{F_{MPR}}} = \frac{\mathrm{F_{B,soil}} \times 0.8}{0.4} = \frac{\mathrm{F_{B,soil}}}{0.5} = 2 \times \mathrm{F_{B,soil}} \qquad (32)$$

Thus, when the bioaccessibility of lead from a particular soil amounts 20%, i.e. $F_{B,soil}$ is 0.20, the relative bioavailability is 0.4. This indicates that for this particular soil introduction of the relative bioavailability in risk assessment causes a reduction in the calculated lead exposure via soil ingestion to 40% compared to the situation where oral bioavailability is not considered. **In general, the calculated lead exposure due to introducing a relative bioavailability is decreased when the bioaccessibility of lead from soil is less than 50%. When bioaccessibility of lead from soil is higher than 50%, a relative bioavailability greater than 1 is obtained.** For the latter case, although not all lead from the soil is bioaccessible, the calculated exposure of a child to lead from soil is higher than for conditions where the MPR_{human} is derived. The reason is that the absorption of lead for MPR_{human}-conditions is for fed conditions, i.e. 40% of dietary lead is absorbed, whereas absorption of bioaccessible lead from soil is assumed to be half of the time for fed and half of the time for fasted conditions.

When the bioaccessibility of lead from soil is determined for both fasted and fed conditions, a weighted value of the bioaccessibility of lead from soil can be used, and the relative bioavailability factor can be determined by:

$$Rel\ \mathrm{F} = 2 \times \frac{\left(\mathrm{F_{B,soil,fasted}} + \mathrm{F_{B,soil,fed}}\right)}{0.5} = \mathrm{F_{B,soil,fasted}} + \mathrm{F_{B,soil,fed}} \qquad (33)$$

9.3 Conclusions

The *in vitro* determined bioaccessibility of lead from soil should be related to a **relative bioavailability factor**, i.e. the factor accounting for the difference in bioavailability of dietary lead and lead from soil. This relative bioavailability factor can be implemented into risk assessment for exposure of lead by a human being via ingestion of soil (and house dust) according to the CSOIL methodology. The derivation of the bioaccessibility and relative bioavailability factor is based on physiology of children.

For the average physiological situation of a child, in which case a child is assumed to be fed for half of the potential play time, and fasted for the other half of the potential play time, the relationship between bioaccessibility determined with the RIVM *in vitro* digestion model and the relative bioavailability factor is:

$$Rel\ \mathrm{F} = \frac{\mathrm{F_{B,soil}}}{0.5}$$

Hence, the calculated lead exposure due to introduction of a relative bioavailability is decreased when the bioaccessibility of lead from soil is less than 50%. When bioaccessibility of lead from soil is higher than 50%, a relative bioavailability greater than 1 is obtained.
For the bioaccessibility of lead from soil the bioaccessibility determined for fasted conditions can be used as a simple but conservative value. For a more realistic value of the bioaccessibility of lead from soil the average bioaccessibility for fasted and fed can be used. However, at the moment, this requires that two different *in vitro* digestion experiments should be performed.

10. Default relative bioavailability factor to be used in the derivation of the Intervention Value

For introduction of oral bioavailability in **general risk assessment** (Intervention Values), a **default relative bioavailability factor** should be determined that is applicable for most of the soils. This default factor can be quantified by determination of the upper bioaccessibility of a certain percentile of soils, e.g. 80% of the soils have a bioaccessibility lower than this bioaccessibility value. Dutch policy makers should choose which boundary should be used for quantification of the default relative bioavailability factor.

Already in 1999 a preliminary default relative bioavailability factor for lead from soil of 0.6 was proposed on the basis of the data available at that time (Lijzen *et al.*, 1999). This was based on the 90th percentile of the bioaccessibility data of historically contaminated soils. At present, many more data are available, which enables the derivation of a default relative bioavailability factor that is a better reflection of the actual situation. Additional information on the relationship between bioaccessibility and soil characteristics is available, whereas also the relationship between bioaccessibility and the relative bioavailability factor is further developed. Finally, the comparison between bioaccessibility results of the RIVM *in vitro* digestion model and *in vivo* bioavailability data of lead from soil was found to be satisfactory (chapter 5). Therefore, in the present chapter, we re-evaluated possibilities for a default relative bioavailability factor.

10.1 Derivation of a default relative bioavailability factor

In order to facilitate the decision-making process of Dutch policy makers for the default relative bioavailability factor, we have derived a number of percentile values for the bioaccessibility of historically contaminated soils with an organic matter content of <20% and >20%, see section 8.2 and Table 7. The reason for incorporating a soil characteristic (organic matter) is that there appears to be a relationship between bioaccessibility and organic matter (Figure 10), and the organic matter content of a soil is almost always known of can be obtained at low costs. The reason to take 20% organic matter was based on expert judgement rather than pure science. Percentile values were determined of the data sets on bioaccessibility values for soils <20% and >20%. These percentile bioaccessibility values were subsequently recalculated to the default relative bioavailability factors which are the upper level for 80, 85, 90, or 95% of the soils. The default relative bioavailability factors are calculated according to (see chapter 9):

$$Rel\ F = \frac{F_{B,soil}}{0.5} \qquad (34)$$

The thus calculated default relative bioavailability factors are presented in Table 7, and they may be adjusted in the future when more bioaccessibility data of historically contaminated soils become available. The present derivation is based on a data set of 25 soils with organic matter content <20% and 18 soils with organic matter content >20%, suggesting that minor changes may occur in the future.
The listed default bioaccessibility factors of lead for soils low in organic matter are close to 1 (0.87-1.20) indicating that for these soils, as a default, little effect on the calculated risk is expected. To be protective in generic risk assessment, and because most soils in the Netherlands are low in organic matter, **we recommend to maintain the present default relative bioavailability factor of "1" for lead.** For specific soil types that are high in organic matter such as "toemaakdekken" a default factor between 0.42 and 0.47, depending on the choice of policy makers, can be used.

Note that the default relative bioavailability factor is larger than 1 for the 95th percentile of the soils low in organic matter. This indicates that some soils show bioaccessibility values that lead to a relative bioavailability factor that is higher than for the MPR-studies. This can be ascribed to derivation of the relationship between *in vitro* bioaccessibility and the relative bioavailability factor, where a weighted value for the absorption of bioaccessible lead is used based on both fasted and fed conditions. A lower absorption of bioaccessible lead is used for the bioavailability of dietary lead (MPR-studies), as fed conditions were assumed.

Table 7. Default bioaccessibility values and relative bioavailability factors associated with different percentiles of the bioaccessibility of lead from historically contaminated soils (bioaccessibility determined for fasted conditions).

Percentile	**Organic matter < 20%**		**Organic matter > 20%**	
	Default **bioaccessibility** value	Default **relative bioavailability factor**	Default **bioaccessibility** value	Default **relative bioavailability factor**
P50	20.4	0.41	10.9	0.22
P80	43.7	0.87	20.8	0.42
P85	43.9	0.88	21.1	0.42
P90	48.6	0.97	21.4	0.43
P95	59.9	1.20	23.3	0.47

The data of soils that were sieved to <50 µm were excluded, as this is not representative for the standard situation. Also bioaccessibility values <1% were excluded as these bioaccessibility values can probably be explained by specific causes, i.e. lead in pottery flakes etc. Averages were used for soils that were tested in duplo, triplo, or on several days, so that each soil was equally important in the derivation of the default bioaccessibility value.
The data were not log-normalized as it is not expected that a specific fraction of all soils would lead to a normal distribution of bioaccessibility values. The derivation of the default bioaccessibility values for organic matter < 20% is based on 25 data points, for organic matter > 20% based on 18 data points.

10.2 Default relative bioavailability value based on all historically contaminated soils

In 1999 a preliminary default relative bioavailability factor of lead from soil of 0.6 was proposed on the basis of all bioaccessibility data of all soils measured up till that time (Lijzen *et al.*, 1999). In section 10.1 default relative bioavailability factors are calculated for soil low (<20%) and high (>20%) in organic matter. **As we think that it is important to consider the information of organic matter, the default values in Table 7 are recommended.**

For comparison with the relative bioavailability factor of 0.6 proposed in 1999, we presently also show the percentile values for the relative bioavailability factors of **all** soils, regardless of the organic matter, see Table 8. To that end, the bioaccessibility values of all soils (43) are used, and the percentile values are calculated both after log-transformation of the bioaccessibility values (parametrical) and without log-transformation (non-parametrical). The bioaccessibility values of all soils have a log-normal distribution. Therefore, log-transformation of the bioaccessibility data and subsequent estimation of the percentile values (parametrical values) is preferred over the non-parametrical values.

Table 8. The 80th, 85th, 90th, and 95th percentile of the bioaccessibility lead of all historically contaminated soils with bioaccessibility >1%, and the corresponding default relative bioavailability factor.

Percentile	Non-parametrical*	Parametrical**	Default relative bioavailability factor$^{\Delta}$
P80	37.9	30.1	0.60
P85	42.9	36.6	0.73
P90	43.8	46.8	0.94
P95	51.0	67.4	1.35

* Non-parametrical quantification of percentiles, i.e. based on unprocessed data set.

** Parametrical quantification of percentiles, i.e. based on log-transformed data set.

$^{\Delta}$ The default relative bioavailability factor is calculated with the parametrically derived percentiles.

As can be seen in Table 8, the 80th percentile of the relative bioavailability factor of all soils is 0.6, e.g. the same factor as proposed as a default in 1999 which was a 90th percentile value (Lijzen *et al.*, 1999). This indicates that the larger number of bioaccessibility values from historically contaminated soil resulted in a somewhat different default relative bioavailability factor. However, considering the organic matter content of the soil as done in section 10.1 resulted in a much larger difference in the recommendation for a default relative bioavailability factor than in the evaluation of 1999. Presently, a default relative bioavailability factor of 1 is recommended for soil low in organic matter, and as most soils in

the Netherlands are low in organic matter, this factor is recommended for generic risk assessment.

10.3 Conclusions

A default relative bioavailability factor can be derived from the bioaccessibility data of lead from historically contaminated soils. This default relative bioavailability factor can be used in **general risk assessment** of soils contaminated with lead, i.e. to change the Intervention Value of lead in soil. The default relative bioavailability factor for soils with an organic matter content of <20% is 0.87, 0.88, 0.97, and 1.20 for the 80^{th}, 85^{th}, 90^{th}, and 95^{th} percentile, respectively. For soils with an organic matter content >20% the default relative bioavailability factors are respectively 0.42, 0.42, 0.43, and 0.47. **Dutch policy makers should decide which percentile should be used for application in risk assessment.**
However, the default relative bioavailability factors for soil low in organic matter (<20%) are all close to 1 (0.87-1.20) indicating that little effect on the calculated exposure of lead is expected. **As most Dutch soils are low in organic matter we recommend to maintain the default relative bioavailability factor of lead from soil of "1".**
The proposal for the default relative bioavailability can be improved in the future if the bioaccessibility of more soils have been determined. In addition, **a further refinement of the default factor can be obtained by using the bioaccessibility for the average physiological situation**, i.e. based on both fasted and fed conditions. This would result in a more realistic and less conservative default relative bioavailability factor. Further research on the bioaccessibility of lead from historically contaminated soils for both fasted and fed conditions is therefore recommended.

11. Use of bioavailability in other countries

This chapter deals with the situation regarding implementing oral bioavailability in soil risk assessment in other countries. The focus is on the USA, UK and Denmark as these countries are up front with research and policy on oral bioavailability of contaminants in soil.

11.1 USA

In the USA the risk of elevated blood lead levels in children (under the age of seven) from environmental lead from different sources is predicted by the Integrated Exposure Uptake Biokinetic (IEUBK) Model (US-EPA, 2002). Media that can act as sources of lead for a child include air, water, soil, dust, diet and other sources (e.g., lead paint). Default media intake rates are recommended for soil/dust and the other sources depending on the age of the child, see Table 9.

Table 9: Default intake rate for total soil and dust ingestion by children according to the US-EPA IEUBK model.

Age child (years)	Default intake rate (g/d)
0-1	0.085
1-4	0.135
4-5	0.100
5-6	0.090
6-7	0.085

The default intake value for total soil and dust ingestion is a ratio of soil ingestion (45%) to dust ingestion (55%).

In the IEUBK model it is assumed that 50% of the lead intake from drinking water and food is absorbed, and that 30% of the lead intake from soil and dust is absorbed. Hence, in the IEUBK model a relative bioavailability of 0.6 (0.3/0.5) for lead in soil and dust is assumed. Results from site-specific studies may be used to change the default absorption values. At present, only bioavailability results from *in vivo* studies with swine have been used, but in the future it might become possible to use also results from *in vitro* tests.

The US-EPA is currently developing a draft Bioavailability Guidance that outlines a generic decision framework on how to assess and incorporate site-specific oral bioavailability adjustments into human health risk assessments at contaminated waste sites (Beringer, 2005;

Maddaloni, 2004). The Guidance focuses specifically on the oral bioavailability of metals in soils. The Guidance Document will address whether a specific *in vitro* bioaccessibility assay has satisfied the validation criteria and can be used to make quantitative site-specific bioavailability adjustments for lead, with certain limitations. The decision framework consists of several stages, with the first using default assumption of oral bioavailability, and decision making whether additional data collection and analysis is likely to improve the site-specific risk assessment. The second stage will provide a process for collecting, analyzing and incorporating additional oral bioavailability into the site-specific risk assessment (Maddaloni, 2004).

The US-EPA has also drafted a document entitled "Estimation of Relative Bioavailability of Lead in Soil and Soil-Like Materials by *in vivo* and *in vitro* Methods", more commonly referred to as the Lead Technical Support Document (US-EPA, 2004). This document does contain a graph showing the correlation between 19 soils where we have both *in vivo* (juvenile swine) and *in vitro* (bioaccessibility) results. Approval of the documents by US-EPA management will likely take several more months. Until these documents are approved, EPA's official position is that only the juvenile swine assay can be used for making site-specific bioavailability adjustments for lead (Beringer, 2005).

11.2 Denmark

In Denmark, bioaccessibility and oral bioavailability are not currently part of the risk assessment of contaminated soil. The Danish EPA, however, is working on the subject. They have asked DHI Water & Environment, Denmark to investigate the possibilities to implement oral bioaccessibility into risk assessment. DHI has implemented and validated the SBRC or Drexler method, a variation of the PBET procedure, and the RIVM *in vitro* digestion model in their present research for the Danish EPA.
DHI recommended in June 2005 to the Danish EPA to correct for reduced oral bioavailability/bioaccessibility in site specific risk assessment (Gron, 2005). More specificaly, DHI recommended endorsing the use of bioaccessibility testing for those contaminants and those test methods that are robust and exhibit proven correlation between *in vivo* and *in vitro* data (Gron, 2005). In practice, the use of bioaccessibility testing is recommended for lead and for cadmium.

11.3 UK

The Environment Agency acts as an advisor to UK government on environmental issues including the assessment of risks to health from land contamination. The Environment

Agency and the Department for Environment, Food and Rural Affairs (Defra) publish guidance documents on dealing with land contamination in England and Wales, and set Soil Guideline Values for different contaminants. Oral bioavailability is recognised as an important factor in the exposure of humans to contaminants and is referred to within the guidance published by the Environment Agency and the Defra. Interest in the UK has been largely focussed on natural arsenic contamination and the usefulness of bioaccessibility determination in the risk assessment of these sites. Whilst there is no formal policy or guidance, the Environment Agency is unable to recommend the use of bioaccessibility testing in human health risk assessment due to the lack of a validated *in vitro* test and the information on robustness and reproducibility of test methods (Environmental Agency, 2005). Practitioners using bioaccessibility testing as part of a risk assessment are advised to treat the data with caution and to provide supporting evidence such as a scientifically robust test method suitable for the contaminant (Environmental Agency, 2005).

In order to further the discussion on bioaccessibility testing, the Environment Agency has commenced a work programme. This includes an international workshop, a bioaccessibility ring test project, in which different bioaccessibility results are compared using the *in vitro* methods of UK laboratories, and the RIVM and US University of Colorado, a literature review and collaboration with overseas regulators and research organisations. Once this programme is completed, the Environment Agency may update its position, if that is appropriate (Environmental Agency, 2005).

11.4 Conclusions

Many countries recognise the importance of oral bioavailability in the estimation of exposure of soil contaminants to humans. Information on the bioavailability of lead from swine studies has been applied in risk assessment in the USA, whereas the use of *in vitro* methods for bioavailability estimation is currently under consideration. In the UK bioaccessibility data of arsenic have been occasionally used in risk assessment, although there is no official recommendation on the use of bioaccessibility in risk assessment. Denmark is considering implementation of information on bioavailability obtained via *in vitro* digestion models in risk assessment.

12. Implementation of oral bioavailability of lead in risk assessment

In the present chapter recommendations are made for the practical implementation of information on oral bioavailability of lead into risk assessment.

12.1 Tiered approach

Information on oral bioavailability of lead from soil can be implemented into risk assessment on different levels with increasing accuracy and, hence, increasing effort needed to perform the assessment. To be able to include oral bioavailability of lead in risk assessment in an efficient way a **tiered approach** is proposed, see Figure 11. Successively, in each tier the degree of conservatism decreases, while site-specificism increases. As a consequence, complexity, and hence effort and finances needed, also increases in each tier. When in a specific tier the human health risk cannot be rejected the assessment in the following tier has to be performed. The underlying principle is: simple when possible - e.g. “no risk” qualification based on a simple procedure in the first tier when there evidently is no risk for human health -, and complex when necessary - e.g. site-specific risk assessment in a higher tier when the risk cannot clearly be rejected and more site-specific details have to be incorporated -.

Ultimately, the (tiered) approach to assess the oral bioavailability should be incorporated in the general procedure on the (tiered) procedure to assess the human health risk due to exposure to contaminated sites.

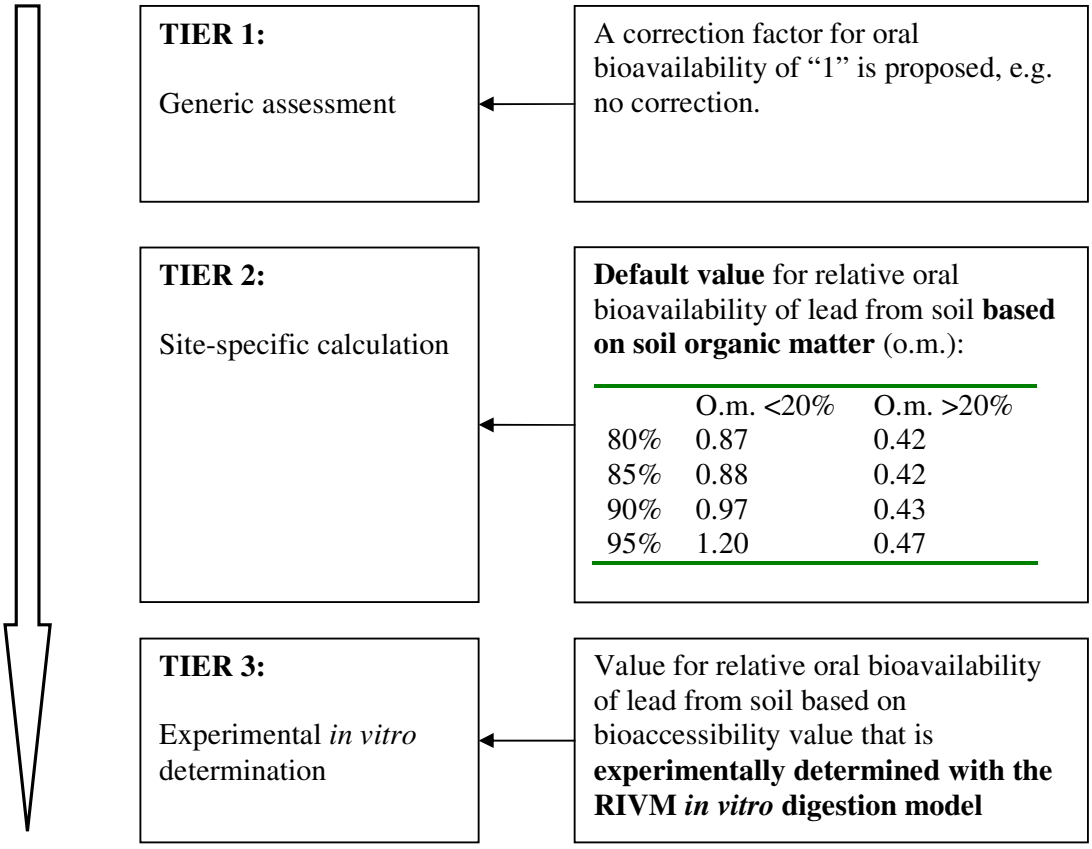

Figure 11. Schematic overview of the implementation of oral bioavailability of lead from soil for different tiers in the risk assessment. The higher the tier the more realistic and less conservative the assessment of oral bioavailability.

The assessment in the different tiers can be described as follows.

In **tier 1** (generic assessment) a **generic** value (i.e. independent on land-use, soil type or soil characteristics) of "1" for the relative oral bioavailability for lead is proposed. In practice, this means that no correction is made for a different bioavailability of lead from soil than from a dietary matrix.

In **tier 2** (site-specific calculation) a more realistic relative oral bioavailability value is calculated through inclusion of soil characteristics. In section 8.2 it was shown that there is a relationship between soil organic matter and the highest bioaccessibility that was measured. However, based on the present data a statistical multiple regression analysis could not be performed. Therefore, for the time being, a pragmatic approach based on visual inspection of Figure 10 is proposed with a default relative bioavailability factor for soils with an organic matter content <20% and >20%. The organic matter content of a soil is a simple soil

characteristic that is always analysed in soil investigation. The 80^{th}, 85^{th}, 90^{th}, and 95^{th} percentile for the default relative bioavailability factor is listed in Table 10 for both organic matter categories. Policy makers should decide which percentile should be used to derive the default relative bioavailability factor that can be used in risk assessment. In practice, the default relative bioavailability factor for soils low in organic matter are close to 1, indicating that application of the default factor in the derivation of Intervention Values has little effect on the outcome. On the other hand, for soil types known to be high in organic matter such as "toemaakdekken" a default relative bioavailability factor of 0.42-0.47 can be used, depending on the choice of policy makers.

In **tier 3**, the bioaccessibility of a soil of a specific site is experimentally determined with the RIVM *in vitro* digestion model. The relationship between the bioaccessibility of lead from soil determined by the RIVM *in vitro* digestion model ($F_{B,soil}$) and the relative bioavailability factor (*Rel* F) is:

$$Rel\ F = \frac{F_{B,soil}}{0.5} \qquad (35)$$

The scientific background for this relationship can be found in chapter 9. For even more realistic estimation of the relative bioavailability factor for the average physiological situation of a child, the bioaccessibility can be determined for both fasted and fed conditions, subsequently averaged, and applied in equation 35.

Table 10. Proposals for default relative bioavailability factors for soils with organic matter content <20% and >20%.

Percentile*	Soils with organic matter <20%	Soils with organic matter >20%
80^{th}	0.87	0.42
85^{th}	0.88	0.42
90^{th}	0.97	0.43
95^{th}	1.20	0.47

* The default relative bioavailability factors are based on the 80^{th}, 85^{th}, 90^{th}, and 95^{th} percentile, indicating that the actual relative bioavailability factor is lower at 80, 85, 90, or 95% of the sites.
Note that in one case the relative bioavailability factor is >1, indicating that the calculated exposure to lead is higher than according to present risk assessment.

12.2 Further research

12.2.1 Short term

Some practical aspects should be given further attention before information on bioavailability of lead from soil as recommended in the present report can be implemented into risk assessment.

- Guidelines should be made for the soil sampling strategy of a contaminated site in order to obtain a representative soil sample for bioaccessibility testing.

- Information and advice should be given to local authorities.

- Depending on the political standpoint, **additional research on a relationship between bioaccessibility of lead from soil for fasted and fed conditions can be performed with the aim to derive a more realistic relative bioavailability factor for the average physiological situation of a child**, e.g. based on an average of fasted and fed conditions for both absorption and bioaccessibility aspects. Based on the present limited data on this issue, it is expected that using bioaccessibility representative for the average physiological situation leads to a lowering of the relative bioavailability factor by about a factor 1.6.

- Contribution to an **ISO (International Standardisation Organisation) standard** on bioaccessibility measurement is recommended. This can be accomplished by the BARGE network, i.e. a group of institutes in Europe that are actively involved in research on the bioavailability and bioaccessibility of soil contaminants. BARGE has connections to the ISO, and the national normalisation institutes. In addition, active involvement in the development on the **unified BARGE method** is recommended. The unified BARGE method is in principle agreed upon by institutes in several European countries (Belgium, Denmark, France, UK, the Netherlands), and also Canada is involved, whereas Germany might join BARGE in the near future. In the beginning of 2006 an interlaboratory study will be performed to investigate the between-laboratory variability and to link the *in vitro* bioaccessibility data to *in vivo* bioavailability data (for arsenic, cadmium, and lead). The RIVM *in vitro* digestion model is used as the basis of the unified BARGE method. The unified BARGE method has the potential to become the standard *in vitro* bioaccessibility test in Europe, and thus contribute to harmonization on soil risk assessment in the EU.

- It has been shown by Oomen and Lijzen *et al.* (2004a) that oral exposure to lead in a residential setting occurs for a major part via direct soil ingestion and via ingestion of **house dust**. Both exposure pathways are almost equally important. Soil is a major fraction of house dust (30-70%), indicating that contaminated soil outside also is

partly responsible for exposure to lead via house dust. In the present report the bioaccessibility of lead from soil has been investigated, and it is implicitly assumed that the bioavailability of lead from soil equals the bioavailability of lead from house dust. However, the bioaccessibility of lead from house dust is unknown. Since house dust is such an important pathway of exposure, we **recommend investigating the bioavailability of lead from house dust**.

- **Reference soil samples** should be prepared, distributed to other institutes within the Netherlands that include bioaccessibility testing into their activities, and controlled. If a relative bioavailability factor is implemented into risk assessment, it is possible that different institutes will include the *in vitro* digestion model in their activities. Hence, in order to pursuit **uniformity**, there is a need for control on the output of the different institutes. Therefore, we advise to provide several reference soil samples that should be used by all institutes, and that should give a bioaccessibility value within a predetermined range. The reference samples should preferably include some Dutch soil samples that are typical for the Dutch situation, and some international samples. The introduction of reference samples is essential as little is known of the inter-laboratory variability.
  Within the BARGE and during a workshop on bioaccessibility in risk assessment in March 2005 in the UK, the need for international reference samples was stressed. In this manner, the outcome of different *in vitro* digestion models can be compared and a comparison between laboratories with the same model is possible.

- And last but not least, contribution to **international harmonization in the framework of the new EU Soil Strategy is recommended**.

12.2.2 Long term

Although not essential for the direct implementation of information on oral bioavailability in risk assessment, the following issues are important to address in further research.

- We recommend to further investigate the **factors that influence the bioaccessibility of lead from soil**, especially for soils with an organic matter content <20%. Bioaccessibility of lead is highly variable for the soils with an organic matter content less than 20%, e.g. bioaccessibility can be less than 1% and higher than 50%. Information on the factors that influence bioaccessibility will lead to more specific default relative bioavailability factors, which will be in most situations <1, i.e. leading to a lower calculated health risk. Also the origin of contamination should be included as a factor that may influence the bioaccessibility. Subsequently, the refined relationships can be implemented in tier 1 or 2 of the risk assessment. In this manner in fewer cases an actual determination of the bioaccessibility by an *in vitro* digestion model is necessary.

- We recommend studying the relationship between *in vivo* bioavailability determined with the juvenile swine method and *in vitro* bioaccessibility determined with 0.6 or 0.2 g of soil per digestion tube, and when necessary with pH adjustment during the *in vitro* digestion procedure. In the present research the gastric pH was in many cases outside the allowed range ($1 < pH < 2$) when 0.6 g of soil per digestion tube was used, resulting in a poor correlation to the *in vivo* situation. A good correlation was found between *in vitro* bioaccessibility and *in vivo* bioavailability when 0.06 g of soil was used per digestion tube in the *in vitro* digestion model, and when the data points with good pH values were used for 0.6 g of soil per digestion tube. By using 0.06 g of soil per digestion tube the pH in the various compartment (stomach, intestine) was almost always within the allowed range (stomach: $1 < pH < 2$; intestine: $5.5 < pH < 6.5$). In addition, with 0.06 g soil per digestion tube, the soil-solution ratio was within the expected physiological range. However, disadvantages of using such a small amount of soil per digestion tube are that 1) the aliquot of soil taken for bioaccessibility determination might not be representative for the entire soil, and 2) this may give rise to difficulties with the detection of lead in the digestive juices. Therefore, using 0.6 or 0.2 g of soil per digestion tube and adjusting the gastric pH when outside the allowed range might be another option.

- A basic assumption in the present proposal for implementation of oral bioavailability in risk assessment is that the health of an average child should be protected. This is based on present views in policy making. However, it is possible that children who display chronic **pica behaviour**, i.e. who deliberately ingest large amounts of soil on a frequent basis, may suffer from lead intoxication. Yet, very little is known on this issue. Oral bioavailability of lead from a large amount of soil may be very low, due to saturation of the contaminant in the digestive juices. Other important gaps of knowledge are 1) the amounts of soil ingested during pica behaviour, 2) the frequency of pica behaviour, and 3) the concurrency of pica behaviour among the children. It is recommended to obtain further insight in pica behaviour so that scientists can quantify the risks of pica behaviour and translate this into practical soil guidelines.

- The present report shows that research on the oral bioavailability and bioaccessibility of a contaminant can have major implications for human health risk assessment. In the case of lead, probably fewer sites will require soil remediation after determination of the bioaccessibility, whereas also from a scientific point of view human health is not compromised. Implementation of specific information on bioavailability may have a large impact on the risk assessment of other soil contaminant. Therefore, we briefly investigate for which compounds a large impact on risk assessment is expected. To that end, 4 aspects should be considered, see also Figure 12:
    - Does the soil contaminant lead to major problems in risk assessment?
    - Is soil ingestion an important pathway of exposure?

- Is the present Intervention Value of the contaminant in soil based on a human health risk, or is an actual risk for humans expected? If the Intervention Value is based on ecotoxicological risks and the contaminant concentration in the soil is not expected to affect human health, research on the oral bioavailability as addressed in the present report is not relevant.
- Is the bioavailability of the compound in the reference toxicity studies expected to be lower than from soil? This can be expected if the matrix used in the reference toxicity studies is water or food, which are expected to give high bioaccessibility of the contaminant, whereas the bioaccessibility of the contaminant from soil is expected to be low.

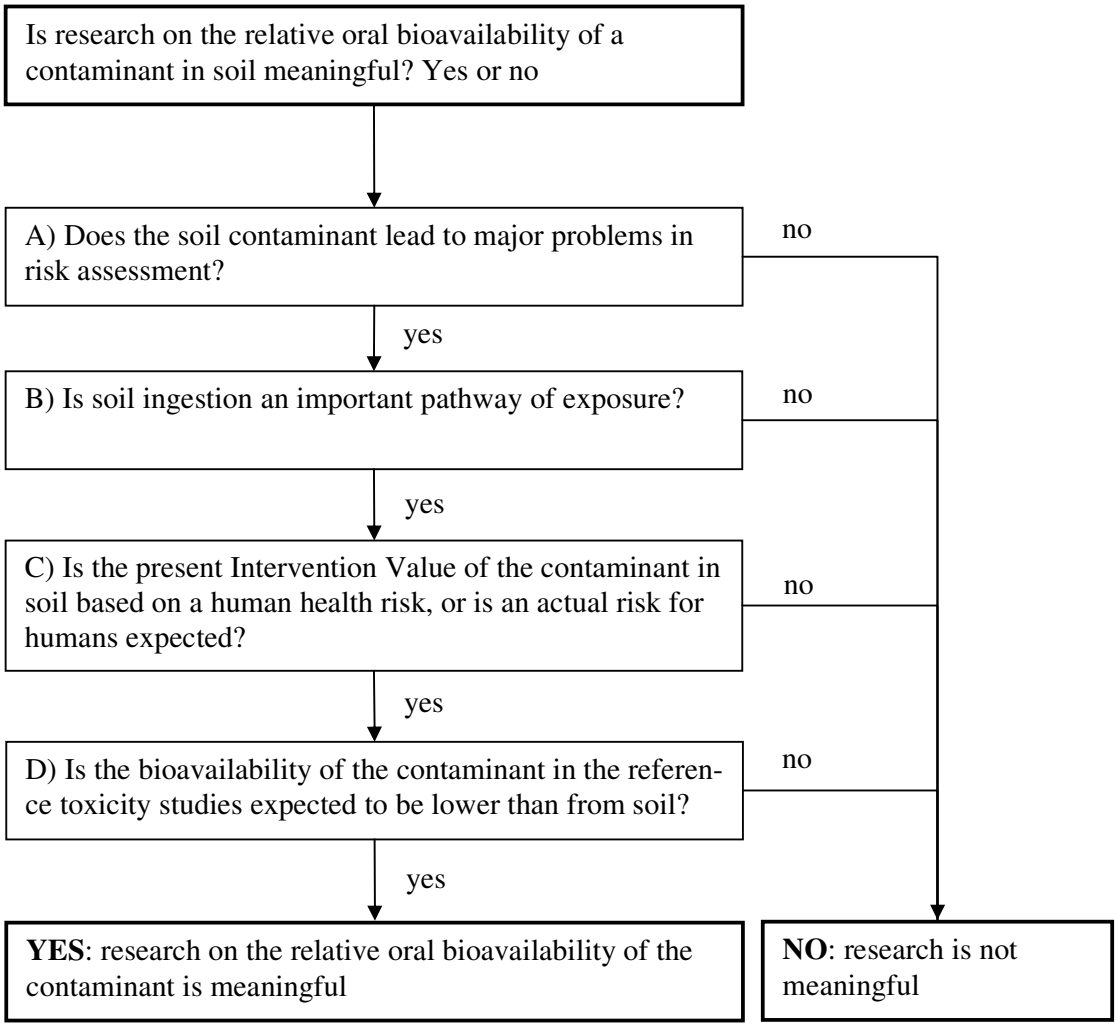

Figure 12. Flow scheme for evaluation whether research on the oral bioavailability and bioaccessibility of a contaminant can have major implications for human health risk assessment.

Within this report a good correlation was shown between *in vitro* bioaccessibility and *in vivo* bioavailability for **arsenic** (section 6.1) and **cadmium** (as determined by DHI, Denmark, see section 6.2). Hence, for these contaminants application of the *in vitro* digestion model for estimation of a relative bioavailability factor seems do-able with relatively little effort. Therefore, we addressed several of the questions listed in Figure 12 resulting in recommendation if further research on oral bioavailability is expected to lead to a considerable reduction in the calculated exposure.

Arsenic. One of the questions in the flow scheme of Figure 12 (question D) is whether the bioavailability of the contaminant in the reference toxicity studies is expected to be lower than for soil. The Tolerable Daily Intake (TDI) of arsenic in the Netherlands is 2.1 µg/kg body weight/day (Baars *et al.*, 2001). This is derived from chronic intake of 100 µg arsenic/l drinking water by humans, assuming a daily intake of drinking water of 1.5 l/day (Baars *et al.*, 2001). Both human studies and studies with experimental animals demonstrate that water-soluble inorganic arsenic compounds are well absorbed after oral intake, up to 95%. Studies of oral absorption of arsenic contaminated dust, soil, and bog ore showed a gastrointestinal absorption of about 10% (Baars *et al.*, 2001). Also the bioaccessibility of the soils with arsenic contamination determined in the present research (section 6.1) show relative bioaccessibility values between 2 and 55%, suggesting that relative bioavailability of arsenic in soil will be considerably lower than 100%. Hence, this question can be answered with "yes".
Another question in the flow scheme of Figure 12 (question B) is whether soil ingestion is an important pathway of exposure. Lijzen *et al.* estimated that soil ingestion accounts for about 71% to the total exposure to arsenic (Lijzen *et al.*, 2001). Hence, also this question can be answered with "yes".
Two other questions addressed in Figure 12 (A and C) should still be addressed to be able to tell whether accounting for a relative oral bioavailability factor of arsenic from soil is expected to have major implications for human health risk assessment. First, it should be investigated whether arsenic contamination is a problem in the Netherlands (question A in Figure 12). Second, it should be investigated whether the present Interventional Value for arsenic in soil is based on a human health risk or risks for humans are expected (question C in Figure 12). **Hence, we recommend a small investigation to answer both questions, and if both questions can be answered positively, we recommend further research for implementation of a relative oral bioavailability factor for arsenic in risk assessment of contaminated soils.** Since the correlation between *in vitro* bioaccessibility and *in vivo* bioavailability of arsenic from soils was satisfactory, the research efforts to come to practical recommendations for application of a relative oral bioavailability factor of arsenic from soil are relatively small.

Cadmium. The answer to the question whether the bioavailability of cadmium in the reference toxicity study is expected to be lower than from soil (question D in Figure 12) is the

following. The oral human-toxicological Maximum Permissible Risk (MPR) for cadmium was set at 1 µg per kg body weight per day (Baars *et al.*, 2001). Oral absorption of cadmium is low, whereas it is likely to depend on physiological status (age, body stores of iron, calcium and zinc, pregnancy, history etc), and also on the presence and levels of ions and other dietary components ingested together with the cadmium compound (Baars *et al.*, 2001). Absorption of cadmium in subjects with low iron was on average 8.9% while those with adequate iron stores absorbed on average 2.3% (Baars *et al.*, 2001). Hence, the low oral bioavailability of cadmium from food and water leaves little room for a much lower bioavailability from soil, and is complicated to determine due to the dependency on the physiological status. Therefore, a very large effect for accounting for relative oral bioavailability is not expected, and a lot of research would be necessary to investigate the best manner to estimate relative oral bioavailability.

In addition, soil ingestion is considered a small pathway of exposure for cadmium for humans, i.e. about 7% according to Lijzen *et al.* (2001) (question B in Figure 12). **Therefore, further research on the bioaccessibility and bioavailability of cadmium from soil is not recommended.**

12.3 Conclusions

For implementation of information on oral bioavailability of lead from soil a **tiered approached** is proposed. In the first tier a correction factor for bioavailability of "1" is proposed, e.g. no correction. If this oral bioavailability value indicates that there is a possible risk, the second tier should be followed. In the second tier additional information on the soil characteristics of a specific site is used to decide whether a default relative bioavailability factor for soil lower or higher than 20% organic matter can be used. The default relative oral bioavailability factor that is applied depends on the political choice. Default relative bioavailability factors for 80, 85, 90, 95% of the soils with <20 and >20% organic matter are listed. For soils with an organic matter content < 20% the relative bioavailability factor for the 80^{th} – 95^{th} percentile of the soils is close to 1, indicating little effect of such a default factor on risk assessment compared to the present situation. In the last tier the bioaccessibility is experimentally determined with the RIVM *in vitro* digestion model so that a site-specific relative bioavailability factor can be calculated. In many cases site-specific determination of the bioaccessibility of lead from soil is expected to lead to a reduction of the calculated risk. Recommendations are made for the short and long term to improve the present knowledge on determination of bioavailability of lead from soil and application into risk assessment. Accounting for the difference in bioavailability of **arsenic** between soil and the matrix used in reference toxicity studies (water) is expected to lead to a considerable reduction in the calculated exposure to arsenic. We therefore recommend a small investigation to determine whether research on the bioavailability of arsenic from soil is worthwhile.

Accounting for the difference in bioavailability of cadmium from food and water versus soil is not expected to lead to a considerable reduction in the calculated exposure to cadmium. No further research on the bioavailability of cadmium from soil is recommended.

13. Conclusions

The present report addresses how information on the bioavailability of soil contaminants in the human body can be obtained and used in human health risk assessment. The research focused on the contaminant **lead** since lead is frequently encountered in the soil at high concentrations and soil ingestion is an important pathway of exposure, especially for children, leading to potential adverse effects.

In the studies that are used as the toxicological basis of the Intervention Value of lead in soil, it was estimated that 40% of the orally ingested lead was bioavailable (see chapter 9). In these studies lead was ingested by children in food and drinking water. However, the bioavailability of a contaminant from soil is mostly lower than the bioavailability of the contaminant from water or food. The difference in bioavailability of a contaminant from soil versus the matrix used in the studies upon which the risk assessment is based can be quantified by the **relative bioavailability factor**. Implementation of the relative bioavailability factor in risk assessment is expected to lead to a more realistic and less conservative estimation of the exposure to a contaminant after soil ingestion.

Below the conclusions of the present research are listed from a practical point of view for policy makers, local authorities and risk assessors (section 13.1), and from a more technical point of view for scientists (section 13.2).

13.1 Conclusions for policy makers, local authorities and risk assessors

- Information on the oral bioavailability of lead from soil can be quantified in a relative bioavailability factor. Implementation of a **relative bioavailability factor** into human health risk assessment of lead-contaminated soils is expected to result in a more realistic exposure assessment. In turn, this leads for lead to a less conservative and more efficient risk assessment, especially in site-specific risk assessment, whereas human health is not compromised.

- **The relative bioavailability factor of lead from soil can be estimated with a simple experimental tool.** The tool, an ***in vitro*** **digestion model**, simulates the conditions in the human gastrointestinal tract. With the *in vitro* digestion model the release of a compound from a matrix (soil, food, water) in the human gastrointestinal tract can be estimated. The release fraction is referred to as the **bioaccessible fraction** of a contaminant. This is a crucial step before a contaminant can become bioavailable.

- The comparison of the bioaccessibility results of lead from soil determined with the RIVM *in vitro* digestion model with *in vivo* bioavailability data of humans (1 soil) and swine (10 soils) was satisfactory. Also some other compounds (arsenic, cadmium, ochratoxin, aflatoxin) show good correlation with *in vivo* data.

- Implementation of a relative bioavailability at different **levels of risk assessment (tiers)** of contaminated soils is proposed (for lead).
    - A correction factor for bioavailability of "1" is proposed for the first tier.
    - In the second tier, a site-specific calculation is performed. A default relative bioavailability factor is proposed for soils with an **organic matter content** <20% and >20%, as the bioaccessibility showed a decreasing trend with increasing organic matter content. The default relative bioavailability factors for 80, 85, 90, or 95% of the soils are presented in Table 11. Which default relative bioavailability factor will be used in risk assessment depends on the political choice. For soils low in organic matter the default relative bioavailability values are close to 1, indicating that for these soil as a default little effect on the calculated risk of lead is expected. On the other hand, for soil types known to be high in organic matter such as "toemaakdekken" a default relative bioavailability factor of 0.42-0.47 can be used, depending on the choice of policy makers.
    - In the third tier, the relative bioavailability factor of lead from soil of a specific site should experimentally be determined by the RIVM *in vitro* digestion model.

Table 11. Proposals for default relative bioavailability factors for soils with organic matter content <20% and >20%.

Percentile*	Soils with organic matter <20%	Soils with organic matter >20%
80th	0.87	0.42
85th	0.88	0.42
90th	0.97	0.43
95th	1.20	0.47

* The default relative bioavailability factors are based on the 80th, 85th, 90th, and 95th percentile, indicating that the actual relative bioavailability factor is lower at 80, 85, 90, or 95% of the sites.
Note that in one case the relative bioavailability factor is >1, indicating that the calculated exposure to lead is higher than according to present risk assessment.

- This relative oral bioavailability factor of a specific soil can be implemented into risk assessment for exposure of lead by a human being via ingestion of soil (and house dust)

according to the **CSOIL methodology, offering an alternative for the present approach in which always a relative oral bioavailability factor of 1 is used.**

- The conditions for which the bioaccessibility should be tested have been studied and are described in the scientific conclusions (section 13.2).

- It was experimentally shown that the lead concentration in soil does not significantly affect the bioaccessibility in the concentration range 0-2650 mg lead per kg dry soil, i.e. up to 5 times the current Intervention Value of 530 mg lead per kg dry soil. Hence, for a particular site with similar soil characteristics, the bioaccessibility (expressed as %) can be assumed to be the same within the concentration range of 0-2650 mg lead per kg dry soil.

- The derivation of the bioaccessibility and relative bioavailability factor is as much as possible based on an "average child". To that end, two important assumptions are made:
    - An average physiological state is assumed for the absorption factor of bioaccessible lead, i.e. a child is half of the potential play time in a fed state and the other half in the fasted state.
    - The amount of soil that is daily ingested by a child is based on hand-to-mouth behaviour, i.e. unintentional transfer of soil by putting fingers in the mouth. However, it is possible that children who display chronic pica behaviour, i.e. who deliberately ingest large amounts of soil on a frequent basis, may suffer from lead intoxication.

- Simulating fasted conditions in the *in vitro* digestion model gives higher lead bioaccessibility values than simulating fed conditions. A human being is part of the time in the fasted and part of the time in a fed state. Therefore, a **weighted value** of the bioaccessibility of lead from soil based on **both fasted and fed conditions** is recommended for estimation of an **average value for oral bioavailability**. Based on the present limited data-set, a relationship between bioaccessibility determined for fasted and fed conditions cannot be derived. However, for a specific soil it is possible to determine the bioaccessibility twice: in an *in vitro* digestion model simulating fasted and fed conditions. Another option is to determine the most conservative bioaccessibility value, e.g. fasted conditions, and use this value to estimate the oral bioavailability of lead from soil. Finally, further research can be performed to estimate the bioaccessibility of an average physiological situation with one experiment. **Policy makers should give directions** in the choice whether a realistic situation should be used in risk assessment, or a cheaper conservative value.

- It is expected that accounting for the relative bioavailability of lead from soil as proposed in the present report leads to a **reduction** in the number of sites contaminated

with lead that require soil remediation. However, with the present knowledge, the bioaccessibility of lead from soil will have to be determined site-specific to account for relative bioavailability. Exceptions are soils known to be high in organic matter, see Table 11.

- The expected costs for determination of the bioaccessibility and the relative oral bioavailability of lead from a specific soil are listed below for various numbers of soils for bioaccessibility determination. As can be seen, the costs per soil decrease when increasing number of soils tested simultaneously. This is because the experimental effort only slightly increases with increasing number of soils.

*Table 12. **Indication** of the costs of location-specific bioaccessibility testing per soil sample (fasted conditions). The bioaccessibility of lead form soil will be determined in duplo, and including a reference and blanc soil in each experimental series. A sampling protocol for soils for bioaccessibility testing will be made in the near future.*

Number of soils	**Costs[1] for bioaccessibility experiment per soil at RIVM**	**Costs[1] for analysis of lead in digestive juice at RIVM (including analysis reference samples, blanc)**
0-5 (0-15)	In mutual agreement: either high costs and rapid bioaccessibility determination, or lower costs and longer period before determination in order to obtain other soils samples to keep down prices	€ 1158 for analysis of up to 5 soils (≥ € 232 per soil)
6-10 (17-25)	€ 525	€ 1586 for analysis of up to 10 soils (≥ € 159 per soil)
11-15 (27-35)	€ 350	€ 2014 for analysis of up to 15 soils (≥ € 134 per soil)
16-20 (37-45)	€ 275	€ 2014 for analysis of up to 20 soils (≥ € 101 per soil)
21-30 (47-65)	€ 200	€ 2593 for analysis of up to 30 soils (≥ € 86 per soil)
> 30	€ 175	In mutual agreement

1) These costs are excluding the pre-treatment of soil, i.e. sampling, drying, sieving, and excluding the costs of analyzing total lead in the soil. These aspects may be determined by the one who commissioned the research, or at RIVM for additional costs. The determination of lead in digestion juice might be performed by the one who commissioned the research, although it should be considered that the specific method for digestion juice is running at RIVM.

- Accounting for the difference in bioavailability of **arsenic** between soil and the matrix used in reference toxicity studies (water) is expected to lead to a considerable reduction in the calculated exposure to arsenic. In addition, soil ingestion is assumed to contribute to about 71% to the total arsenic exposure. We therefore recommend a small investigation to study whether arsenic in the soil is a problem in the Netherlands, and if

risks for humans are expected. If this is the case, we recommend further research for implementation of a relative oral bioavailability factor for arsenic in risk assessment of contaminated soils. Furthermore, since the correlation between *in vitro* bioaccessibility and *in vivo* bioavailability of arsenic from soils was satisfactory, the research efforts to come to practical recommendations for application of a relative oral bioavailability factor of arsenic from soil are relatively small.

13.2 Scientific conclusions

- Bioaccessibility is considered to be an important sub-process of oral bioavailability. Bioaccessibility can be used to estimate the oral bioavailability of a compound from a certain matrix (soil, food, water). Bioaccessibility can be quantified by an *in vitro* digestion model. Advantages of an *in vitro* digestion model to estimate the bioavailability over animal studies are 1) it is a simple, reproducible method, 2) no animals are needed, and 3) it is cheap.

- The bioaccessibility results of the RIVM *in vitro* digestion model have been compared to *in vivo* bioavailability data for lead. The correlation between *in vitro* relative bioaccessibility determined by the RIVM *in vitro* digestion model and relative bioavailability of lead from soil determined in juvenile swine was satisfactory, whereas also the slope of the line was according theoretical expectations. Furthermore, the comparison between *in vitro* bioaccessibility and oral bioavailability of lead from soil for both fasted and fed conditions determined in a human study was good. The predictive value of the RIVM *in vitro* digestion model is also illustrated by the good correlation with *in vivo* data for some other compounds (arsenic, cadmium, ochratoxin, aflatoxin).

- The relationship between the relative bioavailability factor (*Rel* F) and the bioaccessibility ($F_{B,soil}$) is quantified:

  $$Rel\ F = \frac{F_{B,soil}}{0.5}$$

  The derivation of the relationship is based on the average physiological condition of a child, i.e. half of the potential play time fasted conditions and half of the time fed conditions.

- The calculated lead exposure for soil ingestion is decreased due to introduction of a relative bioavailability factor when the bioaccessibility of lead from soil is less than 50%. When bioaccessibility of lead from soil is higher than 50%, a relative bioavailability factor greater than 1 is obtained. In most cases the bioaccessibility of lead from soil is less than 50%.

- We recommend to determine bioaccessibility in the intestinal compartment, as there is a scientific basis for the relationship between intestinal bioaccessibility and bioavailability, as lead absorption occurs primarily in the intestine. Bioaccessibility determined in the stomach compartment results in a physiologically not relevant relative bioavailability factor, which probably overestimates the actual fraction that is available for intestinal absorption for metals. When bioaccessibility is not determined in line with physiology, the probability is higher that the outcome is incorrect for slightly different conditions (different soil types etc).

- We recommend using 0.06 g of soil per digestion tube for the time being. By using such a small amount of soil per digestion tube, the pH during *in vitro* digestion is usually not affected by the soil, so that in almost all cases the results can be used. Also the soil-solution ratio is within the expected ratio in children. However, disadvantages of using such small amounts of soil are that 1) the aliquot of soil taken for bioaccessibility determination might not be representative for the entire soil, and 2) this small amount of soil may give rise to difficulties with the detection of lead in the digestive juices. An option would be to use larger amounts of soil (0.6 or 0.2 g per digestion tube) and adjust the gastric pH if this appears to be outside the allowed range ($1 < pH < 2$). However, before this amount of soil per digestion tube can be applied in risk assessment the correlation with *in vivo* bioavailability data should be checked for soils after pH adjustment.

- The lead concentration in soil does not significantly affect the bioaccessibility in the concentration range 0-2650 mg lead/kg dry soil, i.e. up to 5 times the current Intervention Value of 530 mg lead/kg dry soil. Hence, for a particular site with similar soil characteristics, the bioaccessibility of lead from soil (expressed as %) can be assumed to be the same within the concentration range of 0-2650 mg lead/kg dry soil.

- A trend is observed between bioaccessibility and percentage soil organic matter, with lower bioaccessibility values at high organic matter. The data set of historically contaminated soils is not large enough to identify with multiple regression the soil characteristics that are determining bioaccessibility. Therefore, for the time being, a pragmatic approach is proposed with a default bioaccessibility value for soils of organic matter content <20% and >20%.The default bioaccessibility value can be converted to a default relative bioavailability factor, see Table 11. These default relative bioavailability factors represent an upper estimate of a bioavailability factor that is representative for 80, 85, 90, or 95% of the soils. The default factor for soils low in organic matter is close to 1 (0.87-1.20). This indicates that, as a default, for these soils little effect on the calculated risk is expected. As most soils in the Netherlands are low in organic matter we recommend not to change the present assumption of a default relative bioavailability factor in generic risk assessment (Intervention Value). On the

other hand, for soil types known to be high in organic matter such as “toemaakdekken” a default relative bioavailability factor of 0.42-0.47 can be used, depending on the choice of policy makers. If more bioaccessibility data from historically contaminated soils become available, a reanalysis of the relationship between bioaccessibility and soil characteristics may lead to more precise default relative bioavailability factors.

- A further refinement of the default relative bioavailability factor can be obtained by including data on the bioaccessibility of lead from soil for fed conditions. Further research on the bioaccessibility of lead from historically contaminated soils for both fasted and fed conditions is therefore recommended in order to determine a default relationship between bioaccessibility for fasted and fed conditions, if present. This would probably result in a more realistic and less conservative default relative bioavailability factor.

13.3 Recommendations

Some practical aspects should be given further attention before information on oral bioavailability of lead from soil as recommended in the present report can be implemented into risk assessment.

- Guidelines should be made for the soil sampling strategy of a contaminated site in order to obtain a representative soil sample for bioaccessibility testing.

- Information and advice should be given to local authorities.

- Further research on the relationship between bioaccessibility of lead from soil for fasted and fed conditions is recommended to derive a default relative bioavailability factor that is based on average physiological conditions, i.e. for both absorption and bioaccessibility aspects.

- Contribution to an ISO standard on bioaccessibility measurement is recommended. This can be accomplished by the BARGE network, which has connections to the ISO.

- Active involvement is recommended in the developments of the **unified BARGE method**, for which the RIVM *in vitro* digestion model was used as a basis. The unified BARGE method has the potential to become the European standard.

- Further research on the robustness of the RIVM *in vitro* digestion model is recommended, i.e. between-day variability, interlaboratory variability etc.

- Research on the bioavailability of lead from house dust is recommended. Up until now only one study investigated the bioaccessibility of lead from house dust (Oliver *et al.*, 1999). In a residential setting, oral exposure to lead occurs for a major part via direct soil ingestion and via ingestion of house dust. A primary source of lead in house dust is contaminated soil. In the present report it is implicitly assumed that the bioavailability of lead from soil equals the bioavailability of lead in house dust. In order to investigate the validity of this assumption, further research on this issue is recommended.

- Reference soil samples should be prepared, distributed to other institutes within the Netherlands that include bioaccessibility testing into their activities, and controlled in order to pursuit uniform results in bioaccessibility testing.

- Contribution to international harmonization in the framework of the new EU Soil Strategy is recommended.

In addition, at long terms a few issues are recommended to further investigate.

- It is recommended to further investigate the factors than influence the bioaccessibility of lead from soil. This will lead to more specific default relative bioavailability factors, which will be in most situations <1, i.e. leading to a lower calculated health risk. Subsequently, the refined relationships can be implemented in tier 1 or 2 of the risk assessment. In this manner in fewer cases an actual determination of the bioaccessibility by an *in vitro* digestion model is necessary.

- We recommend studying the relationship between *in vivo* bioavailability determined with the juvenile swine method and *in vitro* bioaccessibility determined with 0.6 or 0.2 g of soil per digestion tube, and when necessary with pH adjustment during the *in vitro* digestion procedure. If this appears to be a good approach a more representative aliquot of soil, potentially leading to less variability, can be used in bioaccessibility testing.

- A basic assumption in the present proposal for implementation of oral bioavailability in risk assessment is that the health of an average child should be protected. This has been a political decision. However, it is possible that children who display chronic **pica behaviour**, i.e. who deliberately ingest large amounts of soil on a frequent basis, may suffer from lead intoxication. Yet, very little is known about this issue. It is recommended to obtain further insight in pica behaviour so that scientists can quantify the risks of pica behaviour and translate this into practical soil guidelines.

- For arsenic oral bioavailability may have major implications for human health risk assessment. We therefore recommend a small investigation to study whether arsenic in the soil is a problem in the Netherlands, and if risks for humans are expected. If this is the case, we recommend further research for implementation of a relative oral bioavailability factor for arsenic in risk assessment of contaminated soils. Furthermore, since the correlation between *in vitro* bioaccessibility and *in vivo* bioavailability of arsenic from soils was satisfactory, the research efforts to come to practical recommendations for application of a relative oral bioavailability factor of arsenic from soil are relatively small.

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